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Contributions to the Genetics of Phaseolus vulgaris.

By

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and

Kiyoo Tabuchi.

With Plates I and II, and one Text-figure.

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Introduction.

A number of papers dealing with the genetics of the qualitative and quantitative characters in *Phaseolus vulgaris* have been published, among which those works contributed by TSCHERMAK,[1] SHULL,[2] EMERSON,[3] SHAW and NORTON,[4] TJEBBES and KOOIMAN,[5] SIRKS,[6] MALINOWSKI,[7] SAX,[8] SAX and McPHEE,[9] KRISTOFFERSON[10] and TAVČAR,[11] may

1) Zeitschr. f. d. landw. Versuchsw. Oesterr., 7: 533–638. 1904. Zeitschr. f. indukt. Abstamm.- u. Vererbungsl., 7: 81–234. 1912. Hereditas, 9 (Festskrift för W. Johannsen): 257–273. 1927.

2) Bot. Gazette, 45: 432–451. 1908.

3) Ann. Rept. Nebr. Agr. Exp. Sta., 22: 67–101. 1909. Nebr. Agr. Exp. Sta. Res. Bull., 7: 3–75. 1916.

4) Mass. Agr. Exp. Sta. Bull., 185: 58–104. 1918.

5) Genetica, 1: 323–346. 1919. Ibid., 3: 28–49. 1921. Ibid., 4: 447–456. 1922.

6) Genetica, 2: 97–114. 1920. Meded. Landbouwhoogeschool Wageningen, 23, 4: 1–40. 1922.

7) Mém. Inst. Gén. Varsovie, 1: 123–178. 1921.

8) Genetics, 8: 552–560. 1923.

9) Jour. Heredity, 14: 205–210. 1923.

10) Hereditas, 5: 33–43. 1924.

11) Zeitschr. f. indukt. Abstamm.- u. Vererbungsl., 40: 83–107. 1925.

be mentioned. The problem is much complicated, or rather confused, especially regarding the seed-coat colour, being due to the difficulty in the classification of the hybrid progeny, in which the segregating forms frequently appear in an almost continuous series of gradation.

For several years the writers have carried on the experiments with *Phaseolus vulgaris*, the results of which will be described in this paper. Owing to the pressure of other work, the present studies are not as complete as they at first had expected. This paper, however, will be published to supply the data so far obtained, rather than to criticize the results of the previous investigators. The naming of the various seed-coat colours is rather difficult, and the accompanying colour plates will assist the identification.

The Stem Colour.

The stem colour exhibits variation, sometimes nearly continuous in gradation. The distinction is much clearer on hypocotyls as well as on cotyledons. The stem colour may roughly be classified into the following three types:

1. Red stem... ... ... coloured intensely (Pl. I, fig. 9).
2. Pink stem ... ... coloured dilutely (Pl. I, fig. 10).
3. Green stem ... ... without anthocyanin pigment (Pl. I, fig. 11).

The genetic relations between these three types are as follows:

Red Stem versus Pink Stem. The F_1 hybrids had red stems by the recessiveness of pink. In Table I are shown F_2 obtained from such F_1 hybrids.

Table I.

F_2 from the cross between red and pink stems.

Cross	Red stem	Pink stem	Total
Y1×15	129	53	182
C2×B6	177	52	229
C1×15	100	32	132
9×Br6	74	15	89
1×7	52	21	73
K4×Br6	72	26	98
Total	604	199	803
Expected	602	201	803

Therefore, red stem behaves as a simple dominant to pink; that is, the two types are exhibited by an allelomorphic pair.

COLOURED STEM VERSUS GREEN STEM. The green stem being recessive to coloured, F_1 obtained had coloured stems; and in F_2, segregation occurred coloured and green stems into a 3 : 1 ratio. The total F_2 data of six crosses amounted to 1056 coloured stems and 312 green stems in number.

PINK STEM VERSUS GREEN STEM. In hybridization of green stem with pink one, the writers obtained F_1 hybrids with red stems, differing from both parents. In Table II are collected F_2 segregated into three types, red, pink and green stems.

TABLE II.

F_2 from the cross between pink and green stems.

Cross	Red stem	Pink stem	Green stem	Total
Bu28×Br3	103	25	32	160
N7×C3	96	31	42	169
W4×C2	67	12	35	114
1×W4	38	11	7	56
Total	304	79	116	499
Expected	281	94	125	500

$\chi^2=4.92$ P=0.086

The segregation is of dihybrid, showing 9 red : 3 pink : 4 green in proportion, therefore two genes are working therein.

RED STEM VERSUS GREEN STEM. Some crosses gave monohybrid segregation for red and green stems, whereas the others gave dihybrid segregation for red, pink and green stems. In Table III are shown results presenting the former assortment.

TABLE III.

F_2 from the cross between red and green stems, showing monohybrid segregation.

Cross	Red stem	Green stem	Total
N7×B2A	436	131	567
W4×B6	60	14	74
Total	496	145	641
Expected	481	160	641

Then, the data indicating dihybrid segregation are collected in Table IV.

TABLE IV.

F_2 from the cross between red and green stems, showing dihybrid segregation.

Cross	Red stem	Pink stem	Green stem	Total
B4 × S3	220	63	88	371
Y2 × B6	12	10	5	27
Total	232	73	93	398
Expected	224	75	99	398

$\chi^2=0.70$ P = 0.725

By these data, we can safely conclude that red is dominant to pink, and each character is concealed in green stems.

COMPLEMENTAL GENES FOR COLOURED STEM. When green stems are intercrossed, F_1 hybrids might be green, but in some crosses the writers obtained coloured F_1. In the latter case, segregation should be followed in F_2. Table V concerns with such an F_2 obtained experimentally.

TABLE V.

F_2 from the intercross of green stems, showing segregation of complemental genes.

Cross	Red stem	Green stem	Total
B4 × Bu28	348	236	584
N7 × 3	61	34	95
Total	409	270	679
Expected	382	297	679

The deviation is somewhat remarkable (27 ± 8.72), but it seems due to the low viability of green stems. In these crosses, no pink stems appeared in F_2.

Now we are able to draw conclusions as to the genetics of the stem colour. By the experimental data here presented, three genes are responsible for the presentation of variation of the stem colour; namely, two genes for green stem and one gene for pink stem. The dominant allelomorphs of the former two genes work as complements in the production of the coloured stems, and neither one by itself can produce them. The gene pink results in pink stem, and its dominant allelomorph manifests red stem, their effects, however, being concealed in green stems.

The Flower Colour.

The flower colour may be roughly grouped under the following four types in the writers' collection:

1. Red flower .. ..coloured intensely (Pl. I, figs. 1-3).
2. Pink flower ... ...coloured dilutely (Pl. I, fig. 4).
3. Flecked flower ... ...anthocyanin flecks occur on white background of wings (Pl. I, fig. 6).
4. White flower... ... ...white (Pl. I, fig. 8).

Within categories of red and pink flowers are exhibited much variation in the intensity itself as well as with or without tint on the base of the standards. Flecks sometimes occur very faintly in colour; and in such a case, the identification is hardly possible. Sometimes too, white flowers are characterized by a very dilute colour, which appears especially on the base of the standard (Pl. I, fig. 7). On account of its diluteness, the recognition is frequently impossible, especially in the hybrid progeny. The crosses containing such flowers as well as the other tinged flowers (Pl. I, fig. 5), therefore, were omitted from the data in the preparation of tables shown below.

Self-coloured flowers bloom on either coloured or green stems. When the stems are coloured, however, the intensity of the flower colour is anticipated by the stem colour; that is, red flowers bloom on red stems, and pink flowers on pink stems. Flecked and white flowers occur invariably on green stems. Therefore, the relations between the colours of stems and flowers are as follows:

Stem colour	Flower colour
Red	Red
Pink	Pink
Green	Red Pink Flecked White

The gene pink being responsible for pink stem as well as pink flower, the pink flower acts as a simple recessive to red, the numerical data accord in part to those shown in Table I. The writers took records on the F_2 populations of crosses, Y1 × 15, C2 × B6 and 9 × Br6, and found 338 red flowers (with red stems) and 116 pink flowers (with pink stems) in total,[1] showing monohybrid segregation for the

[1] Some seedlings died before their flowering time, therefore the number is a little smaller than that given in Table I.

gene *pink*. Owing to the latent nature of pink in white, the white flower may carry either red or pink genotype for the flower colour. When a white flower carrying red genes is crossed with a pink flower, we expect dihybrid segregation in F_2 like that shown by the data in Table II. Observations were made on the flower colour of the F_2 progenies of the two crosses, W4 × C2 and 1 × W4. The F_2 results thus obtained were 105 red flowers (with red stems), 23 pink flowers (with pink stems) and 42 white flowers (with green stems) in total ($\chi^2=3.40$, $P=0.188$). When, however, a white flower carrying red genes is crossed with a red flower, monohybrid segregation will be presented in F_2. The actual data were practically the same as those shown in Table III, therefore we will not cite them here. In the similar matings, *when a white flower carries pink genes instead of red ones, the F_2 results in regard to the flower colour should be dihybrid* as shown in Table IV, because in these cases red stems actually bore red flowers, pink stems bore pink flowers and green stems bore white flowers. When a green-stemmed red flower is crossed with a pink-stemmed pink flower, F_1 is a red-stemmed red flower; and in F_2, segregation occurs in a dihybrid fashion, the data obtained being given in Table VI.

TABLE VI.

F_2 from the cross between green-stemmed red flower and pink-stemmed pink flower.

Cross	Red stem red flower	Pink stem pink flower	Green stem red flower	Green stem pink flower	Total
Bu28 × Br3	103	25	25	7	160
N7 × C3	96	31	29	13	169
Total	199	56	54	20	329
Expected	185	62	62	21	330

$\chi^2=2.72$ $P=0.442$

The results indicate that the gene pink is effective on the flower colour of plants having green stems.

The genetics of the flecked flowers was studied with the progenies of two crosses, B4 × Bu28 and B4 × S3. In the former cross, the partner of the flecked parent was a green-stemmed red flower, whereas in the latter it was a pink-stemmed pink flower. In both cases, F_1 hybrids were red-stemmed red flowers. Tables VII and VIII contain the respective F_2 results obtained from these hybrids.

TABLE VII.

F_2 from the cross between flecked flower and green-stemmed red flower.

Cross	Red stem red flower	Green stem red flower	Flecked flower	Total
B4 × Bu28	338	96	125	559
Expected	314	105	140	559

$\chi^2=4.21$ P=0.124

TABLE VIII.

F_2 from the cross between flecked flower and pink-stemmed pink flower.

Cross	Red stem red flower	Pink stem pink flower	Flecked flower	Total
B4×S3	220	63	88	371
Expected	209	70	93	372

$\chi^2=1.35$ P=0.523

Thus, the flecked flower behaves as a simple recessive to the coloured flower. Owing to the small amount of variegation on the flowers, the identification as to the segregation in the colour intensity is difficult, therefore the two types, red flecked and pink flecked flowers, were included together in Table VIII.

A white flower when crossed with a red-stemmed red flower gave F_1 showing the characters of the latter parent, and in F_2 we obtained the segregating data as presented in Table IX.

TABLE IX.

F_2 from the cross between white flower and red-stemmed red flower.

Cross	Red stem red flower	Green stem red flower	White flower	Total
N7×3	33	13	11	57
Expected	32	11	14	57

$\chi^2=1.04$ P=0.597

The deficit in the white flowers seems due to their low viability. The segregating aspect shows that the dominant allelomorph of the green-stemmed self-coloured flower is complemental with that of the white flower (with green stem) in the production of the coloured stem.

The Seed-Coat Pattern.

On account of the complexity of the variation in the seed-coat colouration, it is advisable to present at first the pattern characters and then the colour inheritance. The pattern characters exhibited on the seed-coat are roughly classified into the following six types:

1. Self-coloured ... ...fully coloured (Pl. II, figs. 12–14, 17–20, 24, 25, 27, 29 and 32).
2. Piebald ... ...partially coloured on white background; modifiers working (Pl. II, figs. 16 (mottled) and 26 (speckled)).
3. Speckled ... ...speckles occurring on white background of spotted; only visible on spotted beans (Pl. II, fig. 26).
4. *Mottled* ... ...*mottled by various colours on dilutely coloured* background (Pl. II, figs. 15, 21–23, 28, 30, 31 and 33).
5. Cream ... ...uniformly coloured being a background colour (Pl. II, figs. 35 and 36).
6. White ... ...white (Pl. II, fig. 34).

The genic analysis with relation to these pattern types presented as follows:

PIEBALD. Piebald acts as a simple recessive character to the self-coloured. In F_2 obtained by the cross between piebald and self-coloured, segregation occurred as shown by the data in Table X.

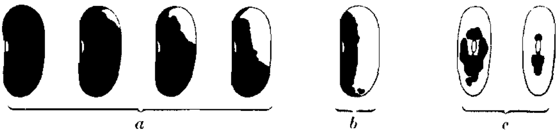
TABLE X.

F_2 from the cross between piebald and self-coloured beans.

Cross	Self-coloured	Piebald	Total
Y2×B6	20	5	25
K4×B4	23	8	31
B4×Y1	8	3	11
Total	51	16	67
Expected	50	17	67

The simple recessiveness of piebald to self-coloured is thus elucidated by the numerical data. Two piebald beans when crossed gave piebald F_1, and the hybrids bred true to piebald in F_2. Strictly speaking, however, one (B4) of the parental beans was greatly spotted or "bald" in type, in which colour distribution occurs on more than a half part of the seed-coat; and the other (S3) was a little spotted or

"saddle" in type, in which colour extension occurs on less than half of it. The F_1 beans were "bald" in type, but the amount of the colour distribution was a little greater compared to the parental "bald." In F_2, segregation took place for the quantity of spotting, giving the three main forms, "bald", "saddle" and "eye" (Text-fig. 1). In this case, the "bald" type includes a considerable series of gradation in the amount of the colour extending over the testa. Apparently the self-coloured beans occurred too. The other two types, "saddle" and "eye", showed a little variation within the respective categories. In Table XI are presented the numerical data obtained in F_2.



Text-fig. 1. Variation in piebald beans. a, "bald"; b, "saddle"; and c, "eye".

TABLE XI.

F_2 from the cross between two piebald beans, "saddle" and "bald".

Cross	"Bald"	"Saddle"	"Eye"	Total
B4×S3	261	56	21	338
Expected	254	63	21	338

$\chi^2=0.97$ P=0.618

The figures indicate that the segregation gave 12 "bald", 3 "saddle" and 1 "eye" in proportion. Aside from the minor variations exhibited within the respective three forms, two qualifying genes must be concerned as regards the partial extension of the colour in this cross.

In another cross observed there was quite a different ratio in the segregation of the three types cited above. When "bald" strain, B4, was crossed with mottled strain, Bu28, the F_1 beans obtained were mottled; and in F_2, segregation took place as indicated by the data in Table XII.

TABLE XII.

F_2 from the cross between "bald" and mottled beans.

Cross	Mottled	Self-coloured	Mottled "bald"	Mottled "saddle"	Mottled "eye"	"Bald"	"Saddle"	"Eye"	Total
B4×Ba28	266	104	49	17	24	20	5	7	492
Expected	277	92	52	17	23	17	6	8	492

$\chi^2=1.60$ P=0.974

The segregating genes are mottled, piebald and two modifiers for piebald. The contents of the piebald segregates including mottled piebald (Pl. II, fig. 16) are 69 "bald", 22 "saddle" and 31 "eye", the numbers corresponding to a 9:3:4 ratio instead of a 12:3:1 as recognized in the cross mentioned just before. The difference in the segregating proportions between the two crosses may indicate that another different modifier is working in the latter cross.

SPECKLED. Piebald strain S3 (Pl. II, fig. 26) has speckles on the colourless part of the testa. Crossing it with a plain piebald bean (B4), speckled F_1 hybrids were obtained; and in F_2, segregation occurred as shown by the data in Table XIII.

TABLE XIII.

F_2 from the cross between speckled piebald and plain piebald beans.

Cross	Speckled	Plain	Total
B4×S3	244	85	329
Expected	247	82	329

From this total, the self-coloured or nearly self-coloured beans, 9 in number, were omitted, because of uncertainty of the presence of speckling. The segregation nearly corresponds to a simple Mendelian ratio, demonstrating that speckled is the dominant allelomorph of the plain condition.

MOTTLED. Mottled character behaves frequently as a simple dominant to self-coloured condition. In such a case, when piebald is crossed with mottled, F_1 should be mottled, and segregation will occur into 9 mottled: 3 self-coloured: 3 mottled piebald: 1 piebald in F_2. The actual data obtained by such a crossing are shown in Table XIV.

TABLE XIV.

F_2 from the cross between piebald and mottled beans.

Cross	Mottled	Self-coloured	Mottled piebald	Piebald	Total
B4 × Bu28	266	104	90	32	492
Expected	277	92	92	31	492

$\chi^2=2.08 \quad P=0.558$

In this cross, two genes, mottled and piebald, are concerned in segregation. On intercrossing self-coloured beans, self-coloured F_1 was obtained in certain crosses; but in a cross mottled F_1 was secured, and segregation occurred in F_2 as shown by the data in Table XV.

TABLE XV.

F_2 of mottled F_1 from the intercross of self-coloured beans.

Cross	Mottled	Self-coloured	Mottled piebald	Piebald	Total
B4 × N6	58	64	23	8	153

The segregating numbers for the mottled character are 81 mottled and 72 non-mottled in total, giving nearly an 1:1 ratio. With these results, the mottled character here appeared by crossing corresponds to its so-called "inconstant" form studied by several investigators. The inconstancy of the form is due presumably to the close linkage of two dominant genes, which act as complements in the manifestation of the mottling.

CREAM. In a cross made between cream and self-coloured beans, F_1 was mottled, and in F_2 occurred segregation into mottled, self-coloured and cream, the data being collected in Table XVI.

TABLE XVI.

F_2 from the cross between cream and self-coloured beans.

Cross	Mottled	Self-coloured	Cream	Total
N7 × B2A	273	111	121	505
Expected	253	126	126	505

$\chi^2=3.57 \quad P=0.173$

The three forms appeared in approaching a 2:1:1 ratio, demonstrating the occurrence of close repulsion. The linkage relation was verified by the F_3 data, which are summarized in Table XVII.

TABLE XVII.

F_3 of cross N7×B2A, showing segregation of mottled and cream.

F_2 parent	No. families	Mottled	Self-coloured	Cream	Total
Mottled	4	32	—	17	49
	61	616	322	269	1207
Self-coloured	30	—	577	—	577
	1	—	7	4	11
Cream	27	—	—	609	609

Of these families tested for their progenies, 61 made segregation dihybridly into 616 mottled, 322 self-coloured and 269 cream in total, the data being that of close repulsion. Aside from the cream families, in which the segregation of mottled cannot be visible on account of the latent nature of mottled in cream, let us consider the origination of the mottled and self-coloured families. From the genotypical analysis, the total 96 families are classified as to their origination, as follows:

Original combination × original combination 91
Recombination × original combination 5

To give such families the following gametes should be needed:

Original combination 187
Recombination 5

Therefore, the approximate frequency of recombination between mottled and cream is 2.6 per cent. In the above calculation, the writers regarded all mottled families segregating into mottled and cream, 4 in number, as due to the union of two gametes, one resulted from the recombination of the linked genes under discussion, and the other from their original combination. In case the doubly heterozygous zygotes were produced by the fertilization of the two recombination gametes, the segregation may be practically the same as in the cases above mentioned, when the numbers are so small as actually observed. But the occurrence of the latter must be relatively very rare compared with that of the former. Therefore, the writers regarded them as such in the calculation.

When cream beans were intercrossed, mottled F_1 hybrids were obtained; and in F_2, segregation for mottled, self-coloured and cream occurred, as indicated by the data in Table XVIII.

TABLE XVIII.

F_2 from the intercross of cream beans having different constitutions.

Cross	Mottled	Self-coloured	Cream	Total
N7×C3	66	29	68	163
Br3×9	39	18	50	107
Total	105	47	118	270
Expected	101	51	118	270

$\chi^2=0.47 \quad P=0.815$

The segregation for non-cream *versus* cream exhibits 152 of the former and 118 of the latter, the numbers being quite in accordance with the expectation made on the basis of a 9:7 ratio. This indicates the occurrence of two complemental genes for the production of the non-cream seeds. The 2:1 ratio of the mottled and self-coloured segregates is due to the fact that one of cream genes is linked with mottled. Therefore, two mottled genes and one of cream genes are linked together very closely, constituting a linkage group.

WHITE. White is a simple recessive to the coloured. In F_2 obtained by the cross between white and mottled, segregation occurred as shown by the data in Table XIX.

TABLE XIX.

F_2 from the cross between white and mottled beans.

Cross	Mottled	Self-coloured	White	Total
W4×ZA	49	24	26	99
Expected	56	19	25	100

$\chi^2=2.20 \quad P=0.339$

The segregating data nearly corresponds to a 9:3:4 ratio. The genes related in this cross are mottled and white. When cream beans were crossed with white beans, the writers obtained mottled F_1 hybrids, and in F_2 the segregation occurred into mottled, cream and

white in one case, and mottled, self-coloured, cream and white in the other. The data giving such results are collected in Tables XX and XXI respectively.

TABLE XX.

F_2 from the cross between white and cream beans, giving mottled, cream and white segregates.

Cross	Mottled	Cream	White	Total
X7×28	38	19	18	75
Expected	42	14	19	75

$\chi^2=2.22$ P=0.336

TABLE XXI.

F_2 from the cross between white and cream beans, giving mottled, self-coloured, cream and white segregates.

Cross	Mottled	Self-coloured	Cream	White	Total
W4×C2	40	21	18	35	114
Expected	43	21	21	29	114

$\chi^2=1.88$ P=0.600

In the cross shown in Table XX, the segregating genes are cream and white, both working on the common basis of mottled; whereas in the cross indicated in Table XXI, the simultaneous segregation took place as to the gene mottled.

The Seed-Coat Colour.

The seed colour is most complicated, and frequently a series of continuous, unseparable gradations of the colour occurs in the hybrid progeny. In this paper, the writers will present the data classifying rather broadly, collecting some indistinguishable forms together (for instance; Pl. II, figs. 13 and 14 are included into black). The main seed colours in the writers' collection are black (Pl. II, fig. 12), brown (Pl. II, figs. 17–19), purple (Pl. II, fig. 27), red (Pl. II, figs. 24 and 25), gray (Pl. II, fig. 29) and yellow (Pl. II, fig. 32), besides cream (Pl. II, figs. 35 and 36) and white (Pl. II, fig. 34).

In a cross made between black (B2A) and cream (N7), F_1 was mottled black; and in F_2, segregation occurred with the frequency distribution as shown in Table XXII.

TABLE XXII.

F_2 from the cross between cream and black beans.

Cross	Mottled black	Mottled brown	Mottled purple	Black	Brown	Purple	Cream	Total
N7×B2A–1	22	4	3	8	2	0	14	53
–2	21	12	0	9	3	2	15	62
–3	24	9	0	12	4	0	13	62
–4	25	8	1	7	3	0	15	59
–5	30	3	2	13	4	0	19	71
–6	41	4	2	9	5	0	7	68
–7	28	8	1	6	6	0	17	66
–8	14	9	2	13	5	0	21	64
Total	205	57	11	77	32	2	121	505

Thus the segregation gave black, brown, purple and cream forms as regards the seed-coat colour; namely, 280 black, 89 brown, 13 purple and 121 cream in total. In Table XXIII are collected the F_3 data of this cross.

TABLE XXIII.

F_3 of cross N7×B2A, showing segregation of black, brown, purple and cream.

F_2 parent	No. families	Black	Brown	Purple	Cream	Total
Black	7	119	—	—	—	119
	9	145	27	—	—	172
	22	282	—	—	71	353
	3	54	14	5	—	73
	1	17	—	3	7	27
	19	225	49	—	87	361
	2	24	3	2	7	36
Brown	2	—	33	—	—	33
	8	—	106	35	—	141
	10	—	132	—	43	175
	2	—	122	30	58	210
Purple	1	—	—	34	—	34
	4	—	—	90	17	107
Cream	27	—	—	—	609	609

The low ratio of the purple segregates in some families is due probably to the difficulty of their identification. Roughly speaking, the segregation accords with a trihybrid assortment for brown, purple and cream.

In Family No. 2, a plant producing yellow seed occurred in addition to those collected in Table XXII, and its progeny contained 16 yellow and 6 cream. The original variant producing yellow seeds had pink stems among the otherwise red or green stems in the family. This may show its mutated origin. The yellow segregates in its progeny had pink stems like their mother plant, and the cream segregates had green stems. In Table XXIV are shown the F_2 data obtained by intercrossing cream beans.

TABLE XXIV.

F_2 from the intercross of cream beans, giving two types of cream.

Cross	Mottled black-purple	Self-coloured black-purple	Grayish cream	Yellowish cream	Total
N7×C3	66	29	51	17	163
Br3×9	39	18	39	11	107
Total	105	47	90	28	270

In the mottled class, purplish seeds appeared predominantly in number compared with blackish ones, whereas the relation was reversed in the self-coloured class. In cream segregates, grayish beans (Pl. II, fig. 35) appeared to be dominant to yellowish ones (Pl. II, fig. 36) in their relative numbers. In another cross, cream × white, the seed colour of F_1 was reddish purple; and in F_2, segregation occurred as shown by the data in Table XXV.

TABLE XXV.

F_2 from the cross between cream and white, giving reddish purple, purple, red, cream and white.

Cross	Reddish purple	Purple	Red	Cream	White	Total
N7×28	30	6	2	19	18	75

The segregation seems to be due to the genes, purple, red, cream and white.

The recessiveness of yellow to brown is demonstrated by observing the data shown in Table XXVI.

Table XXVI.

F_2 from the cross between yellow and black beans.

Cross	Black	Brown	Yellow	Total
Y1×15	121	39	11	171

The segregation seems to occur into 12 black: 3 brown: 1 yellow in proportion. Therefore, yellow is a simple recessive to brown, and both act as hypostatic to black.

When mottled purple was crossed with cream, the F_1 beans obtained were brownish in colour; and in F_2, segregation occurred as indicated by the data collected in Table XXVII.

Table XXVII.

F_2 from the cross between mottled purple and cream beans.

Cross	Mottled brownish	Mottled purple	Mottled red	Mottled yellow	Cream	Total
Bu28×Br3	69	23	8	6	47	153

The brownish segregates include a wide range of variation. In the cross between gray and black, the F_1 beans were black; and in F_2, segregation occurred into black, brown, gray and yellow, the data being shown in Table XXVIII.

Table XXVIII.

F_2 from the cross between gray and black beans.

Cross	Black	Brown	Gray	Yellow	Total
B4×N6	103	34	10	6	153

In another similar cross, the data of which will not be cited on account of the small number observed, the gray segregates appeared about three times the yellow segregates, therefore gray seems to be dominant to yellow in inheritance. By crossing black with red the

writers obtained black F_1 beans; and in F_2, segregation occurred into black, brown-yellow, red and gray, the data being indicated in Table XXIX.

TABLE XXIX.

F_2 from the cross between red and black beans.

Cross	Black	Brown-yellow	Red	Gray	Total
B4 × S3	184	107	21	26	338

The segregating data indicate that the respective genes, red and gray, are recessive to brown. So far as the writers' experiments are concerned, black is always a powerful colour, epistatic to the other colours.

Summary.

1. Two recessive genes are responsible for green stem (Pl. I, fig. 11). The dominant allelomorphs of them work as complements in the production of the coloured stem (Pl. I, figs. 9 and 10), and neither one alone is effective, remaining as green. Pink stem (Pl. I, fig. 10) behaves as a simple recessive to red stem (Pl. 1, fig. 9).

2. Red stems bear invariably red flowers (Pl. 1, figs. 1–3); pink stems bear pink flowers (Pl. 1, fig. 4); and green stems may bear either red, pink, flecked (Pl. I, fig. 6) or white (Pl. I, fig. 8) flowers. Therefore, the gene pink is effective on the flower colour of plants having green stems. Two complemental genes for the production of coloured stems are detectable in the crossing between flecked or white flowers (both having always green stems) and green-stemmed coloured flowers. Flecked and white flowers act as simple recessive characters to the self-coloured condition.

3. Piebald bean (Pl. II, fig. 16 (mottled)) is manifested by a recessive gene, the dominant allelomorph of which is responsible for self-coloured (Pl. II, fig. 12 and others). Three possible modifiers affecting the amount of partial extension (Text-fig. 1) of the colour in the piebald beans are analyzed. Speckled character (Pl. II, fig. 26) behaves as a dominant to the plain condition in inheritance.

4. Mottled bean (Pl. II, fig. 15 and others) acts as simple dominant in its hybrid progeny. The so-called "inconstant" mottling, due presumably to close linkage of two dominant mottled genes, is

also obtained by intercrossing self-coloured beans.

5. The recessive gene cream is linked with the gene mottled, the approximate frequency of recombination due to crossing over being 2.6 per cent. Therefore, these two genes constitute a linkage group with the other mottled gene. Another, different cream gene occurs. The two dominant allelomorphs of these cream genes act as complements in the production of non-cream (mottled or self-coloured) beans. White bean (Pl. II, fig. 34) behaves as a simple recessive to coloured bean. The manifestation of the gene mottled is latent in cream (Pl. II, figs. 35 and 36) and white beans.

6. The most powerful seed-coat colour in inheritance is black (Pl. II, fig. 12), to which brown (Pl. II, figs. 17–19), purple (Pl. II, fig. 27), red (Pl. II, figs. 24 and 25), gray (Pl. II, fig. 29) and yellow (Pl. II, fig. 32) are hypostatic. Brown is in the next rank. The others, purple, red, gray and yellow beans, seem to be manifested by the respective genes. These colours are concealed in cream beans as well as white beans. Grayish cream (Pl. II, fig. 35) is dominant to yellowish one (Pl. II, fig. 36).

EXPLANATION OF PLATES I AND II.

PLATE I.

Figs. 1–8 are drawn in 3/2 natural size and Figs. 9–11 in natural size, and are slightly reduced in reproduction.

Figs. 1–3. Red flowers.
Fig. 4. Pink flower.
Fig. 5. Tinged flower.
Fig. 6. Flecked flower.
Fig. 7. White flower with tinged standard.
Fig. 8. White flower.
Fig. 9. Seedling with red hypocotyl.
Fig. 10. Seedling with pink hypocotyl.
Fig. 11. Seedling with green hypocotyl.

PLATE II.

All the figures are drawn in 3/2 natural size.
Fig. 12. Black bean.
Fig. 13. Brownish black bean.
Fig. 14. Purplish black bean.

Fig. 15. Black mottled bean.
Fig. 16. Black mottled piebald bean.
Figs. 17–19. Brown beans.
Fig. 20. Yellowish brown bean.
Fig. 21. Brown mottled bean.
Fig. 22. Reddish brown mottled bean.
Fig. 23. Yellowish brown mottled bean.
Figs. 24–25. Red beans.
Fig. 26. Red speckled piebald bean.
Fig. 27. Purple bean.
Fig. 28. Purple mottled bean.
Fig. 29. Gray bean.
Figs. 30–31. Gray mottled beans.
Fig. 32. Yellow bean.
Fig. 33. Yellow mottled bean.
Fig. 34. White bean.
Fig. 35. Grayish cream bean.
Fig. 36. Yellowish cream bean.

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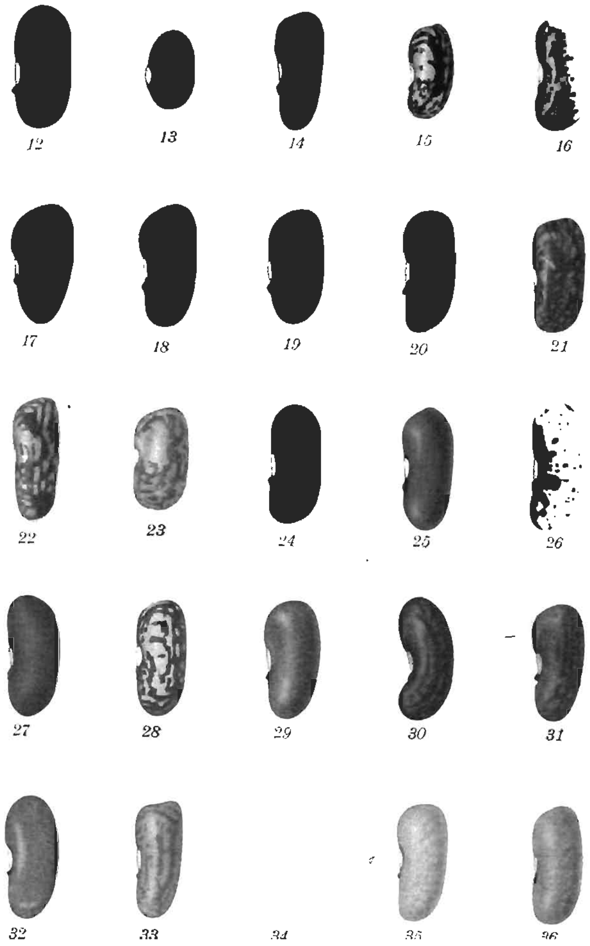
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Genetical Studies on Quamoclit.

By

Sigeroku Nohara.

With Plates III–VII and two Text-figures.

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Introduction.

We have now three species of the genus *Quamoclit* as our ornamental plants. They are *Q. coccinea,* MOENCH, *Q. pennata,* BOJER and *Q. Sloteri,* HOUSE. The former two have been cultivated for a long time in Japan, but *Q. Sloteri* has only recently been introduced and is now spread widely throughout the country.

It was nearly fifteen years ago when I began the genetical studies on the flower color of *Q. pennata,* and in those days the results of many experiments made by others as well as by myself with other plants have shown the complete dominance of one character over the other, so that I was not a little interested then, though the matter is trite now, in finding out such an instance of the intermediate character of flower color as in CORRENS's *Mirabilis Jalapa* (CORRENS, 1912).

I crossed, then, *Q. pennata* with *Q. coccinea* and obtained F_1 hybrid with many characters intermediate between the two species. In external appearance the hybrid was very much like *Q. Sloteri* being somewhat slender compared with the latter.

Since then I was not able to continue the work for various reasons and a long time has passed, but recently I renewed the same work along with other experiments and brought it to a conclusion now. The present paper comprises the results of all the experiments hitherto made.

Materials and Methods.

In the early stages of my experiments *Q. coccinea* and *Q. pennata* were used. The latter species comprises two different forms, viz. the one has a dark "Rose Red" flower (RIDGWAY, 1912) and the other a white, so that I have designated the former A-form and the latter B-form for the sake of convenience throughout the present work.

The seeds of *Q. coccinea* and *Q. pennata* were procured from a seedsman, and those of *Q. Sloteri*, which were not so abundant then, were given me by Mr. IMAI, to whom I am much obliged.

The general characters of these plants are described more or less in many works of systematic botany and of those BAILEY's book (1924) gives somewhat detailed diagnosis, but the characters to be treated in this work are as herein mentioned.

1. *Quamoclit coccinea*, MOENCH.

Cotyledon: At the growth of the stem about 20 cm. high each one of the cotyledons is forked, diverging at 50–80 degrees but 73 degrees on an average of 50 individuals were actually observed; each lobe of a cotyledon 1–2 cm. long (Pl. III, fig. 1; Pl. IV, fig. 9c).

Stem: Colored in dark purple.

Foliage leaves: Petiole attains 10 cm. long or more; blade sometimes longer, heart shaped; margin almost entire sometimes having a few blunt points, each one of which contains a lateral vein. Those points on the margin are assumed to represent the lobes or pinnae of the blade, and the frequency and its statistical constants of such pinnae are

$$M=3.30\pm0.15 \qquad \sigma=1.47\pm0.10 \qquad v=44.55\pm3.72\%$$

and they can be seen in Tables I and II.

TABLE 1.

Variation of leaf-pinnae.

Frequencies		V	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	Totals
pennata-A	obs.																		1	1	1	2	3	5	5	8	12	16	17	21	27	20	6	4	1	150
	%																		1	1	1	1	2	3	3	5	8	11	11	14	18	13	4	3	1	100
coccinea	obs.		6	49	38	29	14	10	3	1																										150
	%		4	33	25	19	9	7	2	1																										100
coccinea × *pennata*-A F_1	obs.									8	21	38	42	75	36	22	5	3																		250
	%									3	8	15	17	30	15	9	2	1																		100
Sloteri	obs.								3	27	36	120	74	155	51	22	9	2	1																	500
	%								1	5	7	24	15	31	10	4	2	1																		100
Sloteri × *coccinea* F_1	obs.								15	29	36	30	21	7	6	4	2																			150
	%								10	19	24	20	14	5	4	3	1																			100

Table II.

Statistical constants in variation of leaf-pinnae based on Table I.

	pennata-A	*coccinea*	*coccinea* × *pennata*-A F_1	*Sloteri*	*Sloteri* × *coccinea* F_1
M	27.11 ± 0.31	3.30 ± 0.15	11.60 ± 0.16	11.19 ± 0.17	9.61 ± 0.18
σ	3.09 ± 0.22	1.47 ± 0.10	1.63 ± 0.12	1.65 ± 0.12	1.78 ± 0.13
c	11.40 ± 0.82	44.55 ± 3.72	14.05 ± 1.01	14.75 ± 1.06	18.52 ± 1.35

The frequencies are not, as we see in Table 1, of the same number but they are all reduced to 100 so as to have the same footing when they are plotted in curves and compared. The variability is expressed in percentage.

In order to obtain the leaf-factor of this species I picked 35 leaves from one plant, 15 leaves from another and calculated the mean of those leaves together and obtained M=1.11.

Peduncle: Several flowered, pedicel erect while having open flower but bent down after fertilization.

Flower: Sepals awn-like pointed; corolla nearly salver-shaped with a long tube; border of corolla nearly 5-angled or the part corresponding to each petal is only curved outwards in outline; color "Scarlet" due to the presence of two distinct pigments mixed together, the one is a color of cell-sap tinged with a pigment "La France Pink" and the other is that of cytoplasmic substances tinged with "Yellow Ocher" pigment as seen under microscope. The cytoplasmic substances are of irregular shape and undoubtedly represent chromoplasts.

Pollen: Spherical in shape, having fine projections on the outer wall as is shown in Pl. VI, fig. 15. The size of the pollen grains was measured and the results are shown in Table III.

In Table III we see that the majority of the pollen grains is nearly of the same size excepting those of the hybrid between *Q. coccinea* and *Q. pennata* A-form, and the variability of the pollen-size of this plant is 7.23±0.42% as shown by its coefficient.

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TABLE III.

Frequency table of pollen-size.

v	*coccinea*	*pennata*–A	*pennata*–B	*pennata*–A × B F_1	*coccinea* × *pennata*–A F_1
3					3
4					16
5					18
6					29
7					45
8	6	3	6	6	25
9	34	31	36	35	6
10	93	95	90	92	4
11	15	13	14	13	3
12	2	8	4	4	1
n	150	150	150	150	150

The number under *v* represents the scale number of ocular micrometer and the unit corresponds to 17.5 μ.

TABLE IV.

Statistical constants of pollen-size based on Table III.

	coccinea	*pennata*–A	*pennata*–B	*pennata*–A × B, F_1	*coccinea* × *pennata*–A, F_1
M	9.82±0.06	9.95±0.06	9.83±0.06	9.83±0.06	6.66±0.14
σ	0.71±0.04	0.76±0.04	0.75±0.04	0.75±0.04	1.70±0.10
v	7.23±0.42	7.64±0.44	7.63±0.44	7.63±0.44	25.53±1.52

Errors are calculated in mean-error and *v* is represented in %.

Fruit: 4-celled, 1-seeded in each cell, points downwards as it ripens as is already stated.

Seed: Shorter in comparison with those of other species.

2. *Quamoclit pennata*, BOJER.

A-form.

Cotyledon: Petiole mostly 8–15 mm. long when the plant grows about 20 cm. high; lobes forked at 100–160 degrees, average degrees being 118.4 measuring 20 individuals from both A-form and B-form; each cotyledon-lobe 25–45 mm. long or more, having two conspicuous veins as in the foregoing one (Pl. III, fig. 2).

Stem: Colored with dark purple but this color varies more or less in intensity according to the conditions of exposure to the sun.

Foliage leaves: Petiole 8–15 mm. long or more; blade pinnately parted into slender, almost filiform divisions, most frequent number being 29, and each division has a lateral vein (Pl. IV, figs. 6, 9 a). Statistical constants of the number of pinnae are

$$M=27.11\pm0.31 \qquad \sigma=3.09\pm0.22 \qquad v=11.40\pm0.82\%$$

(see Tables I and II).

I took 28 leaves from one plant of this form and 22 from another one, calculating the leaf-factor by adding them together and I obtained 0.98 as the mean.

Peduncle: 1 or 2 flowered, pedicel upright throughout the life time.

Flower: Salver-shaped but 5-lobed corolla with dark "Rose Red" color, due to the presence of the dilute "Rose Red" cell-sap as seen under microscope.

Pollen: Shape and size about the same as those of the foregoing one as is shown in Pl. VI, fig. 14 and in Tables III and IV.

Fruit: 4-celled, 1-seeded in each cell, upright throughout the life time, as is shown in Pl. IV, fig. 6.

Seed: About twice as long as that of the foregoing one.

3. *Quamoclit pennata*, Bojer.

B-form.

All appearances of this form are about the same as A-form with only the exceptions in color of the corolla which is almost white but tinged with very dilute greenish yellow, and the absence of purple color on the stem or any part of the plant body.

The differences between statistical constants of the pollen-size, as we see in Tables III and IV, are very slight and they are of course due to fluctuation.

4. *Quamoclit Sloteri*, House.

The plant resembles somewhat the F_1-plant of the hybrid between *Q. coccinea* and *Q. pennata* A-form as a whole, but a close examination reveals at once that the plant is different from the hybrid in many respects; therefore the characters of the plant will be mentioned later comparing it with F_1-plant of the hybrid.

The methods of the experiments are as follows:

As the flowers of the plants I used are all entomophilous I covered them with bags made of parchment paper whenever self- or cross-fertilization was wanted.

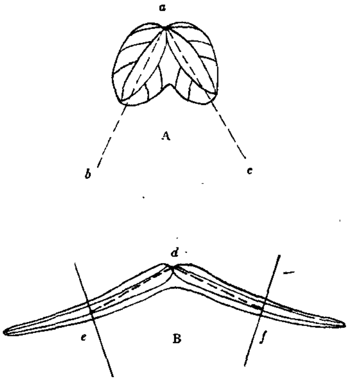
Castration and artificial pollination were performed as usual.

Self-fertilized seeds of both *Q. coccinea* and *Q. pennata* can easily be obtained in the ordinary way and crossed seeds between the two forms of the latter (A-form and B-form) and the seeds of F_1- and subsequent generations also can be easily obtained in the same way.

To obtain the seeds of interspecific crosses, however, is very hard so hard that only a few fruits can be obtained, each containing one or at most two seeds, out of a hundred trials in the ordinary way.

I found, however, that when we do not use the paper bags for interspecific crosses a little greater percentage of seeds could be obtained, perhaps owing to less unusual conditions within the envelope of the bags. In doing this I selected only one individual among the fewer plants for the female parent and destroyed all the rest of the same species. In pollinating no bags were used as is stated above, although there is some danger of their being naturally crossed with undesired pollen.

The seeds thus obtained, however, can be distinguished from others after the seedlings have developed their cotyledons or foliage leaves. But such extraordinary methods can only be applied when we want some crossed seeds irrespective of the numerical ratio.



Text-fig. 1. Schema showing how to measure the divergence of cotyledon-lobes.

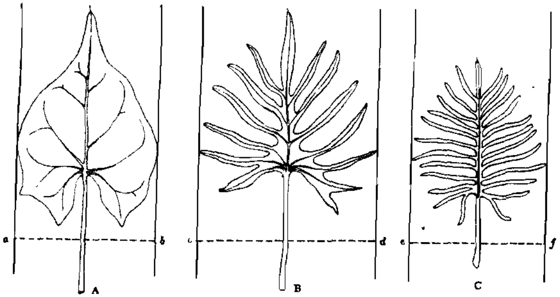
Seeds from F_1-individuals which were raised by interspecific crossing have never been obtained in any way as far as my experiments are concerned.

The method of measuring the divergence of cotyledon-lobes is to take the angle made by the lines as *ba* and *ca* of A, or the lines *ed* and *fd* of B in Text-fig. 1. The lines *ba* and *ca* are drawn from the distal end of the lobe to the base of the cotyledon, but when the lobes are curved as in B the lines are drawn from the middle of the lobes to the base of the cotyledon as is shown in *ed* and *fd* in B.

The proportion between the length and the breadth of the leaves is almost constant in the same species or race, therefore this ratio may be considered as a characteristic of the species or race.

LEAKE (1911) has investigated the inheritance of the so-called leaf-factor in cotton. His leaf-factor is essentially a length-breadth index of the terminal lobe of the leaf. LEAKE's leaf-factor, however, can only be used in plants with palmate leaf as in cotton. So I have used here as the leaf-factor the value obtained by dividing the length of the blade by its breadth.

In measuring the leaf-length I took the length from the base to the apex of the blade, and as the leaf-breadth I took the widest distance between the two lateral sides as *ab* in Text-fig. 2, A, and when the leaf is pinnately parted as is that of *Q. pennata* I took the distance between the two parallel lines which are drawn along the two



Text-fig. 2. Showing the method of measuring leaf-breadth.

lateral sides of that outline as *cd* and *ef* in Text-fig. 2, B and C respectively.

In counting the pinnae, the one lobe or pinna which contains a mid-rib is ignored, and in the leaves of *Q. coccinea* a serra of the margin is taken as a pinna excepting the one with a mid-rib.

Although I do not examine the nature of the seeds it is almost apparent that there is a fact, in some kinds of *Quamoclit* seeds, comparable to the so called "Hartschaligkeit" found most commonly among the seeds of *Astragalus sinensis, Phaseolus Mungo,* var. *subtrilobata,* so that some seeds of *Quamoclit* remain intact without showing any signs of germination for more than 120 days under the ground.

In order to assist the seeds to germinate at once I used to grind the seeds gently with fine quartz sand in a mortar or to grate an end of the seed on the coarse surface of a brick in order to cut the seed-coat, taking care not to injure the embryo.

Other particular methods, when employed, will be mentioned under the respective section.

Examination of Pedigrees of Parent-Stocks.

I obtained some seeds of *Q. coccinea* and *Q. pennata* A-form in 1912 and next year *Q. pennata* B-form was added. In 1913 they were grown in good conditions and all bloomed in due time.

On examination they were found to be similar in all external appearances to their respective parents and to each other among their sister plants.

Again in 1914 quite a number of seeds of those three forms which were obtained by self-pollination the year before were sown and grown in good condition, and on examination they proved to be true to their types so that their pedigrees will be genetically pure as regards the characters in question.

A few seeds of *Q. Sloteri* coming from Mr. IMAI were sown and I obtained 35 selfed seeds in 1915. Next year they were sown and 18 individuals were grown to bloom. The characters of the offspring were similar to those of their parents and they also were all alike among their sister plants as far as the external characters are concerned. Moreover not only the offspring raised from selfed seeds were all alike but also F_1-plants produced in hybridizing *Sloteri* to *coccinea* were all alike as will be shown later so that the *Sloteri*-plants in my possession are of a pure line as far as the external characters are concerned.

Crossing.

1. A-form × B-form of *Q. pennata.*

Quamoclit pennata A-form and B-form were first used for hybrid making and the hybridization was carried on without any difficulty.

The allelomorphic pairs in these forms are the colors of "Red" vs. "White" of the flowers as well as "Purple" vs. "Green" of the stem.

F_1-generation: Ten seeds of the cross-pollination were obtained in 1913 and they were sown the next year. Eight individuals of F_1-generation have grown to maturity and they were similar with each other among the offspring as is explained in Mendel's law in every respect of the characters in question.

On comparison of the characters of these plants with those of the parents it was found that the only difference is the color of the flowers as well as the stems.

As has already been mentioned the characters of the forms A and B are almost the same excepting the color of the flowers and the stems, so it is natural to expect to have similarity of size, shape of leaves, nature of fruits etc. excepting the difference of color in the plant body as stated above from those of the parents.

The color of the flower in F_1-plants is pinkish as a whole and it looks as if "Rose Red" dye were diluted with water.

The flower is not, however, of homogeneous color but some patches corresponding to the mid-rib of each petal and the part near its margin have a deeper color than the rest. We may say roughly, therefore, that the nature of color inheritance is intermediate between both sides of the parents.

The presence of purple color in the stem is associated with the flower color, and the stem color of an F_1-plant is probably diluted as the flower color is, but its fluctuation is so strong that it makes it difficult to distinguish from that of an A-form.

I tested also the nature of the characters in question on the reciprocal cross to the above one, namely B-form × A-form, and got some plants of F_1-generation which proved to be identical with F_1-plant in the direct cross; so I did not carry on further experiments with the reciprocal one.

F_2-generation: In 1914 I obtained 155 self-fertilized seeds from a

plant of F_1-generation and they were sown the next year, 1915.

The offspring which grew up to bloom were only 131 individuals, that is, the rest did not germinate or died before blossoming.

The mature individuals appeared in the following number:

A-form 31 : F_1-type 65 : B-form 35.

We obtain ± 2.25 for deviation and ± 4.96 for standard error in calculation by the formula $\sigma = \sqrt{npq}$ (YULE 1911), when we take $p = 31 + 65$ and $q = 35$ from the total number 131, assuming the p and the q to make 3:1 ratio. The deviation is much smaller than the other standard error and this shows that the former has no significance than the mere chance due to random sampling.

Again we obtain ± 0.5 for deviation and ± 5.72 for standard error in the same way when we take $p = 66$ and $q = 65$ from the total number, assuming the p and the q to make 1:1 ratio. In this case the deviation is by far smaller than the standard error and this also shows that the deviation has no significance other than mere chance due to random sampling.

I kept one individual from each of these three forms in F_2-generation, i.e., A-form, F_1-form and B-form, and obtained some seeds from them for further investigation.

F_3-generation: In the spring of 1916 I sowed 124 seeds from A-form, 44 seeds from B-form and 186 seeds from F_1-form or intermediate form, the total number being 354.

In that summer they bloomed vigorously with a few exceptions which died in the seedling stage or did not germinate.

Examining the mature individuals we obtained the following results:

A-form 95, all came true to the type.
B-form 35, all came true to the type.
F_1-form 135, split as follows:
A-form 36 : F_1-type 70 : B-form 32.

The last three forms which split in this case make an approximate ratio of 1:2:1 respectively, when we calculate them based on the total number, 138, in the same way as before.

From the above mentioned results we see that the color inheritance follows the simple Mendelian law and there will be no need of further experiments on the character in question. The stem color seems to associate with the flower color as is very often the case.

2. *Q. coccinea* × *Q. pennata* A-form.

The crossings between these two species were made reciprocally, and as the results of both crosses proved to be quite similar, I shall describe here only those of *Q. coccinea* × *Q. pennata* A-form.

An effective cross can not be done so easily as that of the inter-racial crosses, so that I tried over a hundred flowers to get fruits at one season, but only a few were successful.

Afterwards, I devised the method, which was described in the foregoing section, to get fruits more successfully.

F_1-generation: In the spring of 1913 seven seeds of the cross were sown, and the plants had grown in good condition and bloomed prolifically, but no fruits were produced.

The characters of F_1-plants obtained are as follows:

Cotyledon: Petiole 20–25 mm. long; leaf forked divergent at 70–120 degrees, the mean being 95 degrees. Each lobe 20–27 mm. long, having two conspicuous veins (Pl. III, fig. 3).

Stem: Colored in dark purple.

Foliage leaves: Petioles attain 60–70 mm. long; blade pinnately parted but not so slender in divisions and the number of pinnae is not so large as that of *Q. pennata* (Pl. IV, figs. 7, 9 b).

I counted the number of pinnae of the leaves of the hybrid and the results thus far obtained are shown in Tables I and II.

Calculating the statistical constants based on Table I we obtain the following:

$$M=11.60\pm0.16 \qquad \sigma=1.63\pm0.12 \qquad \tau=14.05\pm1.01$$

I took 28 leaves from one plant and 22 from its sister plant of this generation, and calculating the leaf-factor I obtained 1.06 as the mean.

Peduncle: Several flowers on a peduncle, each pedicel upright at least during flowering time, but soon after the corolla is shed the pedicel with the ovary falls off, hence it is totally sterile, usually turning to greenish yellow so that the behavior of the pedicel after the flower has withered is not known; in short, whether it bends down toward the maturity of the fruits or not is not known.

Flower: Corolla salver-shaped with a long tube and its border nearly 5-angled; color "Scarlet Red" coming near "Spectrum Red" representing intermediate color between that of the two parents (Pl. IV, fig. 9 b).

This color is not simple but it seems to be due to the presence of two distinct elements when observed under the microscope. The one

is a color of cell-sap as is seen in the flower of *Q. pennata* A-form, and the other is that of chromoplasts similar to that seen in the flower cells of *Q. coccinea*. Then the color elements of these flowers seem to be contributed from both sides of the parents, but red pigment of *Q. pennata* A-form seems to dominate over "Yellow Ocher" of *Q. coccinea*, so that the flowers look red rather than yellow.

Pollen: The majority of the pollen grains is spherical in shape with warty wall; the size is not the same, some are larger attaining 180μ and others are smaller, being 50μ in diameter and sometimes they are not spherical in shape as will be seen in Pl. VI, fig. 16.

The results of measurement of pollen-size are shown in Tables III and IV, and the statistical constants of the pollen size are as follows:

$$M=6.66\pm0.14 \qquad \sigma=1.70\pm0.10 \qquad v=25.53\pm1.52\%$$

When we compare the size and its variability etc. of the pollen grains of *Q. coccinea*, *Q. pennata* A-form, and their hybrid plant respectively we can clearly see that the size and shape of the two former are nearly the same but those of the hybrid are very different, and that the variability of the latter is more than three times that of the former, that is it shows that the size of the pollen grains of the latter is very irregular. Such irregularity of the pollen grains is of course due to the abnormal division of the pollen mother-cells. Correspondingly the female elements or egg-cells can be surmised to be abnormal if not in size or shape but in the cell-elements, because we could not obtain any seeds by back-crossing taking the F_1-plant as female parent.

F_2-generation: As is stated the F_1-plants were found to be quite sterile in self-pollination, so I tried to obtain some seeds by the back-crossing in the following schedule:

(*coccinea* × *pennata*-A) × *pennata*-A
pennata-A × (*coccinea* × *pennata*-A)
(*coccinea* × *pennata*-A) × *coccinea*
coccinea × (*coccinea* × *pennata*-A)
(*coccinea* × *pennata*-A) × *pennata*-B
pennata-B × (*coccinea* × *pennata*-A)

Strictly speaking the last two in this schedule are not back-crossing, but I used B-form by way of an experiment with quite new elements.

All the trials were in vain so that I am inclined to conclude that both the male and the female gametes of the hybrid, i. e. of *coccinea* × *pennata*-A F_1 are totally devoid of fertilizing power. Thus I could not obtain any seeds of F_1-plant either by self-pollination or by back-crossing as far as my efforts were concerned. Accordingly we can not know any thing about the behavior of the F_2-plant.

The characters of the parents and their hybrids are tabulated in Table V.

TABLE V.

	Characters	*coccinea*	Hybrid	*pennata*-A
Cotyledon	Degrees of divergence of two lobes	50–80 M = 73.2	70–120 M = 95.	100–160 M = 118.4
	Length of lobe	10–20 mm.	20–27 mm.	25–45 mm.
Leaf	Mean number of pinnae and their variability in%	M = 3.30 ± 0.15 v = 44.55 ± 3.72	M = 11.60 ± 0.16 v = 14.05 ± 1.01	M = 27.11 ± 0.31 v = 11.40 ± 0.82
	Leaf-factor	1.11	1.06	0.98
Peduncle	Number of flowers	Several	Several	A few
Pedicel	Direction	Erect while blooming but bends down toward maturity of fruits	Upright during at least flowering time but subsequent nature not known	Upright throughout the life time
Flower	Corolla border	5-angled	Nearly 5-angled	5-lobed
	Color	Scarlet	Intermediate	Dark Rose Red
Pollen	Shape	Spherical	Spherical or irregular	Spherical
	Size	M = 9.82 ± 0.06 v = 7.23 ± 0.42 %	M = 6.66 ± 0.14 v = 25.53 ± 1.52%	M = 9.95 ± 0.06 v = 7.64 ± 0.44%
Fruit	Direction	Points downward	Not known	Points upward
Seed	Length	Shorter	Not known	Longer

4. Crossing between *Q. Sloteri* and *Q. coccinea*.

Crossing between *Q. Sloteri* and *Q. coccinea* presents less difficulty than that between *Q. coccinea* and *Q. pennata*, and some seeds were obtained.

The F_1-plants were grown the next year and they came to bloom.

On examination, the characters of F_1-plants were found to be intermediate between those of the two parents in general (see Pl. III, fig. 5; Pl. V, fig. 11.) and they can be seen in Table VI.

TABLE VI.

	Sloteri	*Sloteri* × *coccinea* F_1	*coccinea*
Divergence of cotyledon-lobes	90°-110°	75°-80°	50°-80°
Color of corolla	Pomegranate near Rose Red	Scarlet nearing Scarlet Red which shows the intermediate of both parents. The origin of the color is the same with that of *Sloteri*.	Scarlet
Pollen-size	12.09 as in Pl. VI, fig. 17	10.21 as in Pl. VI, fig. 18	9.82 as in Pl. VI, fig. 15
Leaf-pinnae	Deeply parted as in Pl. V, fig. 13 a.	Less deeply parted as in Pl. V, fig. 13 b.	Almost entire as in Pl. V, fig. 13 c.

As the F_1-plant is quite sterile like that of *coccinea* × *pennata* the behavior of subsequent generations can not be known.

As is already stated the pollen grains of *Q. coccinea* are regularly round and nearly of the same size (Pl. VI, fig. 15) and those of *Q. Sloteri* have the same feature (Pl. VI, fig. 17) but the pollen grains of the hybrid are as irregular in shape and size just as those of the hybrid between *Q. coccinea* and *Q. pennata* are, while those of the parents are regular. Correspondingly the female elements may perhaps hold the same trait and the irregularity of the reproductive elements can also be supposed to be a cause of sterility, being one of the instances of sterility of interspecific hybrid.

The number of individuals of F_1-plants is not large enough to determine the genetical constitution of *Q. Sloteri*, yet it is almost clear that the latter does not segregate in this F_1-generation, which corresponds to the back-crossing, if the plant were a hybrid between *Q. coccinea* and

some other form; in other words *Q. Sloteri* is of a pure type as regards the characters in question.

The Origin of *Q. Sloteri.*

Quamoclit Sloteri is not described in older systematic books, so it seems to have been introduced into popular notice comparatively late, though its existence may be pretty old as we notice it in Mr. BARKER's information which will be mentioned later.

Some years later Dr. MAKINO called my attention to the description of *Q. Sloteri* in BAILEY's manual (1924) and a similar statement was also found in a pocket book by SHAW (1917). The plant, according to the descriptions, seems to be recognized as a hybrid in origin between *Q. coccinea* and *Q. pennata*, but it does not seem to be identical with the F_1-plant of my hybrid of the same species.

When we compare the characters of *Q. Sloteri* with those of F_1-plant of my hybrid, we can notice at once that there is a considerable difference in many respects.

As we see in Pl. VII, fig. 19, the cotyledons, the first few foliage leaves and stem of this plant (*Sloteri* to the left) are a little larger and stouter than the corresponding parts of my hybrid (*coccinea*×*pennata* A-form to the right) but the divergence of the cotyledon-lobes of the former is about the same as that of the latter, though the variability of *Q. Sloteri* is much smaller than the other. Even when fully grown the feature of the characters mentioned above holds the same relation as can be seen in Pl. VII, fig. 20 (*Sloteri* to the left, hybrid to the right).

The leaves of *Q. Sloteri* are broad and the breadth is much larger than the length in contrast to my hybrid as is shown in Pl. VII, fig. 20. When I calculated the leaf-factor I obtained 0.68 on an average of 28 leaves taken from *Q. Sloteri* and 1.06 on 22 leaves from the hybrid. The results also show how the leaves differ.

The flower of *Q. Sloteri* is much larger than that of my hybrid and the outline of the flower border of the former is rather circular while that of the latter is pentagonal as can be seen in Pl. VII, fig. 20. One of the most conspicuous differences of *Q. Sloteri* from the hybrid is that the former is partially fertile while the latter is totally sterile.

Less conspicuous differences are the flower color and the number of pinnae on the leaves. Flower color of *Q. Sloteri* is light "Pome-

granate Purple" nearing "Rose Red" consisting of two elements, red and yellow. The red element is due to the presence of anthocyanin and it dominates over the yellow which is due to the presence of chromoplasts bearing the yellow color "Chamois" of RIDGWAY's Plate XXX, 19.

The number of pinnae of *Q. Sloteri* is like that of the hybrid as is shown in Table II.

I have grown more than 50 plants of *Q. Sloteri* during several generations, but I have never seen among the offspring any other type than *Q. Sloteri* excepting a supposed reversional individual, of which a little more will be written later on.

As can be judged from the above mentioned descriptions of characters we can think that the plants of *Q. Sloteri* under my care are all of a pure line in regard to the characters in question, but can not think that it is identical with the plant of F_1-plant between *Q. coccinea* and *Q. pennata*, because if this be a hybrid it would have segregated during several generations of cultivation as is mentioned above.

Then, what is the origin of *Q. Sloteri* and how did the plant come to exist?

Thinking that America is the native place of *Q. Sloteri* I asked Dr. E. E. BARKER of Cambridge, Mass. to identify the plant and to get any information about its origin sending him a part of my hybrid-plant and a photograph of *Q. Sloteri*. He kindly took the trouble to take them to the Gray Herbarium of Harvard University and showed them to Dr. FERNALD.

In comparing the photograph and my specimen with the preserved ones of the herbarium they found that one of the preserved specimens closely resembles mine and Dr. BARKER wrote to me. The main part of the letter is as follows:

"They have one specimen which closely resembles yours. It is a very old one, having been collected in 1849 and belonged originally in the collection of GEORGE ENGLEMAN of St. Louis. It is labelled *Quamoclit coccinea* × *pennata*. Obviously, it resembles both these species in being intermediate in its leaf form between the two."

"*Quamoclit coccinea* has a variety *heterophylla* which shows many differing forms of leaf. If a cross were made between any of these varieties and *pennata* it is only to be expected that the segregates of such a hybrid would show many differences in types of leaf forms, according to the type of leaf possessed by the *heterophylla* parent. So, if these specimens differ from your hybrids between these species, that is not surprising. Dr. FERNALD concurred with me in considering your photograph and pressed leaves to be from a hybrid between *Quamoclit coccinea* × *Q. pennata*."

If *Q. Sloteri* is not identical with F_1-plant of the hybrid between *coccinea* and *pennata* it might have been derived from some plant like that, perhaps from a hybrid between *heterophylla* and *pennata* as Dr. BARKER suggested. Moreover the occurrence of such a plant is to be regarded as a reversional type, which will be stated in the next section, or at least a modified type from *Q. Sloteri* seems to account for the origin of the plant.

Let us think then how such a stable or fixed form as *Q. Sloteri* came about, but before going to seek the possibility of the existence of such a form let us consider the physical constitution of those plants.

Judged from the stouter and larger features of the plant body of *Q. Sloteri* one may easily be led to imagine that the cells of the plant body are larger than those of the smaller and less stouter plant body of *coccinea* × *pennata* F_1, and that the chromosome number of *Q. Sloteri* would be also larger than that of the other, for there are many instances of this kind. *Oenothera gigas* investigated by DE VRIES and GATES, some species of *Solanum* by WINKLER, *Datura* by BLAKESLEE and BELLING are noted examples.

These giant plants have not only larger organs but the chromosome number is larger than those of the normal plants, in spite of how the giant plants might have arisen.

When we compare the pollen-size of *Q. Sloteri* with that of *Q. coccinea* and *Q. pennata* we see that the former is much larger than the latter as is shown in Tables IV and VI (*Sloteri*=12.09, *coccinea*=9.82, *pennata* A-form=9.95). The same relation may be held by the other parts, although I did not measure the cells of other parts of the plant body.

As to the chromosome number of these plants KANÔ (1929) reports that *Q. Sloteri* has 30 chromosomes in haploid number while *Q. coccinea* and *Q. pennata* have 15, i. e., the first is tetraploid plant. Dr. KAGAWA informed me that he obtained the same results independently. Based on the karyological standpoint of view it is very likely that *Q. Sloteri* has been derived from the other two, and if it is so, this is one of the examples of tetraploidy induced by hybridization just as in the case of *Primula kewensis* investigated by DIGBY (1912).

Next let us turn to consider the possibility of the existence of such a form as *Q. Sloteri*, a tetraploid type.

1. If the hybrid, whether it be an interspecific or an interracial hybrid, can produce abundant seeds there would be a possibility of making such a stable form by extraction among recombinations, but

in the case of the hybrid between *Q. coccinea* and *Q. pennata* the state of things is quite the reverse, consequently *Q. Sloteri* would not have been derived in this way.

2. Although the hybrid is quite sterile now, yet we can not warrant that it will remain the same all the time, for we have such an instance as that of the sterile hybrid of two diploid plants which has transformed into a fertile form, having a tetraploid number of chromosomes, and fixedly maintained the type as in the case of *Primula kewenensis*.

As is already stated the F_1-plant of the hybrid between *Q. coccinea* and *Q. pennata* can be produced by cutting and it will maintain its life for a long time, so it is very likely to produce such a partially sterile plant as *Q. Sloteri* from the hybrid through long culture.

Reversion from Q. *Sloteri*.

In the spring of 1918 I sowed 15 seeds of *Q. Sloteri* got by self-pollination in 1917 and obtained the offspring in good condition.

Among them a peculiar plant appeared suddenly as is mentioned in the previous section and it resembles very much in some respects a plant of the hybrids between *Q. coccinea* and *Q. pennata* on the one side and its sister plants on the other.

From the seedling until the flower time I compared the characters of this plant with those of the plants on both sides and I came to consider the plant to be one reverted to its original type, but it did not yield any seeds.

A few points of the characters of the supposed reverted type and those of others may be seen in Table VII.

TABLE VII.

	Sloteri	Reverted	F_1 hybrid of *coccinea* × *pennata*
Length of corolla	39.8 mm., average of 20	38.2 mm., average of 20	38.5 mm., average of 10
Color of corolla	Pomegranate near Rose Red	Dark Spectrum Red	Scarlet Red near Spectrum Red
Diameter of corolla	26.71 mm., average of 20	24.33 mm., average of 20	22.00 mm., average of 10
Pollen-size in 17.5 μ scale	12.09 average of 50	7.64 average of 50	6.66 average of 150.

As is shown in Table VII the characters of the supposed reverted type are different from those of *Q. Sloteri* and of the hybrid of *coccinea* ×*pennata*, but in some respects the features resemble those of the hybrid rather than *Q. Sloteri;* such as the direction of pedicel during flowering time and the total sterility etc.

To my regret as it is sterile I could not obtain the seeds by self-pollination and I was not able to see the subsequent behavior of the plant.

Discussion.

The only difference in the characters between *Q. pennata* A-form and B-form is the presence of color in the flowers and stem of the former and absence of the same from the latter, and the hybrid characters in question are each displayed by one gene difference.

Now the intermediate characters in hybrids can oftener be observed rather than the cases of perfect dominance, but in earlier days since the rediscovery of Mendelian laws the appearance of the intermediate characters seemed to me to be looked upon as rather a rare case.

CORRENS's *Mirabilis Jalapa* among plants (CORRENS 1912), and Andalusian fowl among animals explained by BATESON and PUNNETT are well known intermediate examples.

My case of flower color in two races of *Q. pennata* is quite similar with that of CORRENS's *Mirabilis* which was, then, regarded to be rather a rare case as is mentioned above.

As is already stated the flower color of *Q. pennata* A-form and that of the hybrid between two forms of *Q. pennata* are associated with the color of the stem. It might be a case of manifold effects of a gene (MORGAN, 1915), but unless it has been verified it may better be said to be a case of the so-called complete linkage of genes.

In regard to the species hybrid a few words more will be added.

As is already stated the pedicel of *Q. pennata* is upright throughout life but that of *Q. coccinea* is erect during flower time only, and after fertilization it bends down, so that the pedicel of F_1-plants of the hybrid is erect during flower time as is expected to be, but the behavior of the pedicel after fertilization can not be known because the plants are sterile.

The nature of the pedicels of both parents after fertilization is quite similar to that of two races of *Capsicum annuum* used in experiments by IKENO (1913), so that it is inferred that the nature of the

pedicel of F_1-plants may have been the same as that of the *Capsicum* hybrid, i. e., after being fertilized the pedicel will bend down to some extent.

The leaf shape of the F_1-plants is intermediate between the parents as in the case of LEAKE's cotton and it may be supposed that in the F_2-generation the shape will display segregation in the ratio of 1:2:1 after the fashion of cotton, but this supposition can not be verified.

The plant of the hybrid can easily be propagated by means of cutting and if the conditions be favorable they will maintain the life for years renewing the activity by propagating young shoots in such a way.

As was already known and also can be seen in Pl. IV, fig. 9 b and Pl. V, fig. 13 b, the characters of F_1-plants between two different species are, in general, intermediate in many respects, so that if we cross one pure type which displays the same characters in question successively as one of the parents to such an intermediate stable mate, generation after generation, the characters in question tend to come near those of the pure type.

Thus, in the case of *Quamoclit* we see that the leaf-shape of *Q. Sloteri* is intermediate of the parents and that of F_1-plant of *Sloteri* × *coccinea* is also intermediate of the parents, but when the leaf-shape of the first hybrid is compared with that of the second, supposing *Q. Sloteri* is the first hybrid form between *coccinea* and *pennata*, we will notice that the shape of the second will come nearer that of one parent, i. e., nearer to *coccinea* than to *pennata* in this case. The principle of the so-called grading in animal breeding seems to follow this.

A complete or a partial sterility is so often met with in species-hybrids that it led some investigators (STANDISH 1916) to think that conversely such phenomenon is an evidence of species-hybrid.

Although the reverse is not always true as there are a good many instances to prove it, yet the case of *Q. Sloteri* might possibly be one of species-hybrid and most cases of such sterility are probably due to the same cause which, according to JESENKO (1913), is ascribed to the inharmonious action of the plasma of both parents.

Summary.

1. The flower color of the hybrid between *Quamoclit pennata* A-form and B-form is intermediate and it is displayed by one gene difference segregating after a simple Mendelian ratio.

2. The stem color of the hybrid is also perhaps intermediate and it is displayed by one gene difference behaving as the flower color does, but the stem color is variable according as it is more exposed or not in the sun, in addition to the interference with the green ground color of the stem.

3. The gene of the stem color of A-form and that of the flower may almost completely be linked, but it might be a case of manifold effects of a gene.

4. The characters of the species-hybrid between *Q. coccinea* and *Q. pennata* A-form are mostly intermediate between those of both parents as a whole.

5. The F_1-plant of this species-hybrid does not yield any fruits as in most other cases, so that the behavior of the subsequent generations can not be known.

6. *Q. Sloteri* is now a fixed race probably derived from a hybrid between *Q. coccinea* and *Q. pennata* or their varieties.

7. The F_1-plant of *coccinea* × *pennata* is completely sterile but a partially fertile hybrid as *Q. Sloteri* is assumed to have arisen by internal change or mutation on the hybrid during a long culture, otherwise it might have arisen from a hybrid produced between varieties of one parent or both.

8. From *Q. Sloteri* once occurred a reversional type which resembles an F_1-plant of *coccinea* × *pennata*, the fact being one evidence showing that the above mentioned assumption is highly probable.

9. F_1-plant of the hybrid between *Q. Sloteri* and *Q. coccinea* displayed the intermediate characters of the parents as a whole and there appeared no plant identical with either *Q. Sloteri* or *Q. coccinea*.

10. The hybrid between *Q. Sloteri* and *Q. coccinea* was also quite sterile as in the case of *coccinea* × *pennata*.

In closing I wish to express my sincere thanks to Prof. IKENO for his kind advice given on the work. I wish also to thank Drs. MAKINO, IMAI, BARKER and FERNALD who were very kind in various ways, and Messrs. TOPPATA and SAITÔ who were very helpful to me in the field work during the present investigation.

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EXPLANATION OF PLATES III—VII.

Plate III.

Fig. 1. Seedling of *Q. coccinea*. About natural size.

Fig. 2. Seedling of *Q. pennata*. About natural size.

Fig. 3. Seedling of *coccinea* × *pennata* F_1. About natural size.

Fig. 4. Seedling of *Q. Sloteri*. About natural size.

Fig. 5. Seedling of *Sloteri* × *coccinea* F_1. About natural size.

Plate IV.

Fig. 6. Mature plant of *Q. pennata*. Very much reduced.

Fig. 7. Mature plant of *pennata* × *coccinea* F_1. Very much reduced.

Fig. 8. Mature plant of *Q. coccinea*. Very much reduced.

Fig. 9. Parts of flowers, foliage leaves and cotyledon of those plants shown in figs. 6, 7 and 8 of this Plate. a, b and c are taken from plants of figs. 6, 7 and 8 respectively.

Plate V.

Fig. 10. Mature plant of *Q. Sloteri*. Very much reduced.

Fig. 11. Mature plant of *Sloteri* × *coccinea* F_1. Very much reduced.

Fig. 12. Mature plant of *Q. coccinea*. Same plant as Pl. IV, fig. 8. Very much reduced.

Fig. 13. a, b and c—Parts of flowers and foliage leaves taken from plants shown figs. 10, 11 and 12 of this Plate respectively.

Plate VI.

Fig. 14. Pollen of *Q. pennata*-A. × ca. 230.

Fig. 15. Pollen of *Q. coccinea*. × ca. 230.

Fig. 16. Pollen of *coccinea* × *pennata* F_1. × ca. 230.

Fig. 17. Pollen of *Q. Sloteri*. × ca. 230.

Fig. 18. Pollen of *Sloteri* × *coccinea* F_1. × ca. 230.

Plate VII.

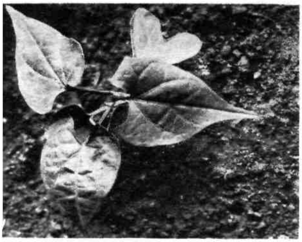
(All about natural size)

Fig. 19. Seedling of *Q. Sloteri* to the left and seedling of *coccinea* × *pennata* F_1 to the right.

Fig. 20. Part of *Q. Sloteri* to the left* and part of *coccinea* × *pennata* F_1 to the right.

Fig. 21. Part of *Sloteri* × *coccinea* F_1.

* The part is taken from near the top of the plant so that the leaves are comparatively small.



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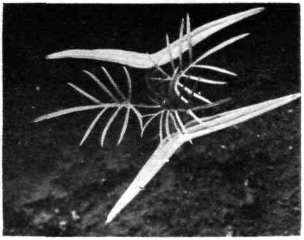

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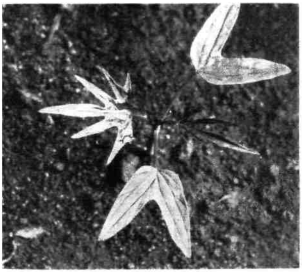

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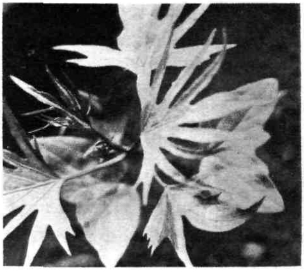

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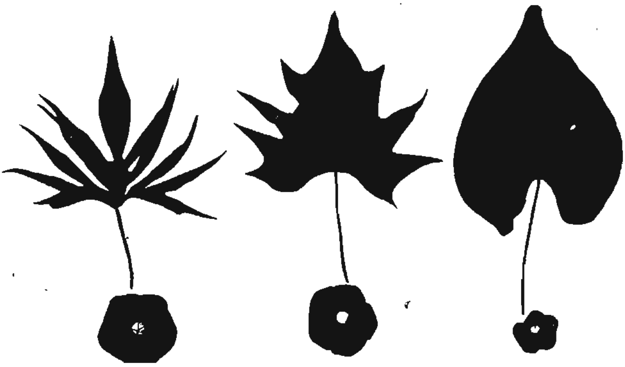

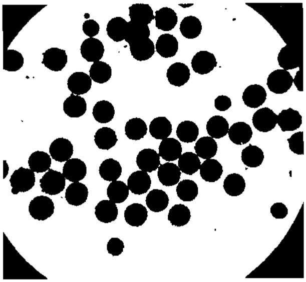

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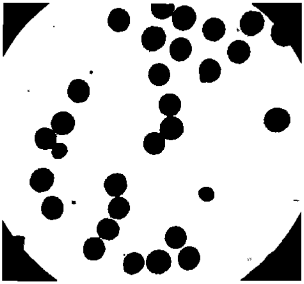

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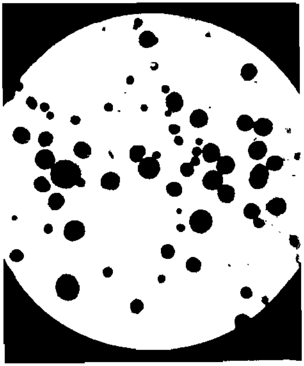

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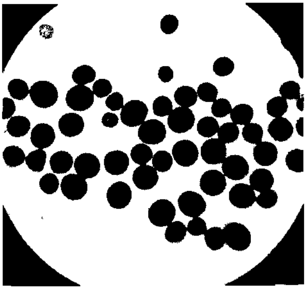

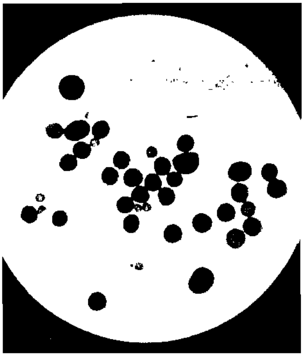

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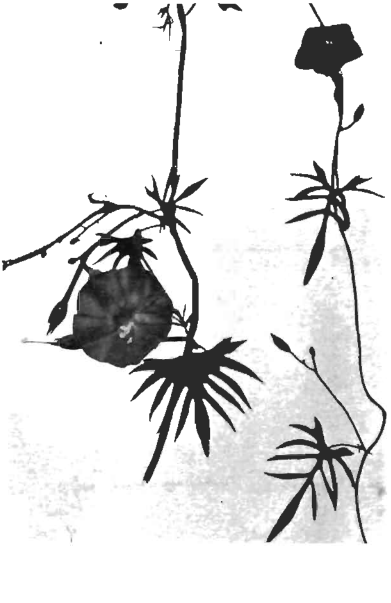

The Effect of Respiratory Injury on the Silk Gland and Sexual Organ in the Silkworm, Bombyx mori L.

By

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With four Figures in the Text.

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Introduction.

In the insect the tracheal branches are closely distributed in various tissues and organs so that respiratory injury exercises a paramount influence upon their development and function. However, very little has hitherto been reported on this respect. Under these circumstances an attempt was made to elucidate whether respiratory injury affects the development and function of the silk gland and sexual organ in the silkworm. Upon this the present account is based.

Here I deem it my duty to tender my hearty thanks to Dr. S. Ishiwata and Professor T. Kaburaki for advice and criticism. My thanks are also due to Dr. J. Machida for assistance generously given.

Material and Methods.

For experiments, the fifth stage larvae of the following univoltin, multivoltin and some other races were used: "Nichi No. 1," "Nichi No. 1"×"Shi No. 4," "Sekigi," "Daizo," etc.

Up to the present the majority of investigators have caused respiratory injury by closing many spiracles on both sides with wax, butter, starch, gelatin, enamel paint, and others. On account of its remarkable influences upon various physiological changes of body fluid, pulsation, muscular paralysis, as well as appetite and growth, however, this procedure is regarded to be inadequate to the present investigation. Accordingly an operation was made on one side of the body by pressing gelatin into the fourth spiracle for the middle division of the silk gland and the sixth spiracle for its posterior division and the sexual organ, to make a contrast to the condition found on the unoperated side, where any compensation, so far as observations went, could not be traced at all. In order to make out the actual effect on the posterior division of the silk gland, it may be necessary to operate on four spiracles from the fourth to the seventh. However, such an operation exercises a serious influence on various physiological processes of the worm body; consequently it appears to me to be not suitable for examination. Here observations were restricted to noting the effect of respiratory injury upon the sexual organ as well as upon the middle division and a small part of the posterior division of the silk gland.

The larvae moderately operated on soon began to take food and presented less difference in the physiological condition and days required for maturation as compared with these unoperated.

Observations.

1. *Preliminary Investigation.*

As a preliminary investigation, some experiments were carried out to elucidate the relation between the size of the spiracle and the development of the silk gland and sexual organ in the normal larvae, with the following results (12, 13). 1) The development of the silk gland always stands in positive correlation with the size of the fourth spiracle at the middle of the fifth larval stage, although this correlation is disturbed in the full-grown stage by the abrupt development of the gland. 2) The size of the ovary and testis is also in positive correlation with that of the sixth spiracle throughout the course of the fifth stage, although not so completely as in the case of the silk gland. However, in the imaginal stage the number of the eggs found in the egg tubes on both sides scarcely correlates with the size of the

spiracles in the larval stage. 3) The rate of asymmetry of the silk glands on both sides is extremely variable according to stages, as is shown in Table I. 4) The rate of asymmetry of the sexual organs in the larvae cannot be determined so exactly as in the late pupal and imaginal stages of the female, which have the rate of asymmetry variable according to orientations or positions in the pupal stage, as is shown in Table II.

TABLE I.

The rate of asymmetry of the silk glands on both sides at various stages.

5th larval stage	No. of cases	Rate of asymmetry (%)		
		max.	min.	mean
1.5 day	19	17.0	0.4	5.6
6th day	16	7.0	0.4	2.5
8th day	20	37.0	0.0	7.8

TABLE II.

The rate of asymmetry of the number of eggs found in the egg-tubes of both sides according to orientations or positions in the pupal stages.

Orientation or position in pupal stage.	No. of cases	Number of eggs			Rate of asymmetry (%)		
		max.	min.	mean	max.	min.	mean
Laid down normally	20	865	592	690.6	5.50	0.00	1.771
Laid on the right side	18	753	523	638.7	17.78	0.45	4.572
Erected	19	810	582	661.4	5.50	0.33	2.419
Inverted	20	782	573	662.4	1.83	0.14	0.941

2. THE SILK GLAND.

The fourth spiracle on one side was operated on the second day of the fifth larval stage, and the gland was dissected out on maturation for examination. In twenty four individuals fairly well operated on the gland on the operated side was always smaller than that on the opposite unoperated side. The operation on the fourth spiracle affects not only the middle division, but also the posterior division of the gland, though invariably more remarkable in the former than in the

latter. Therefore the percentage of the weight of the middle division to the total weight of the gland on the operated side is much smaller than that on the unoperated side, as is shown in Table III.

TABLE III.

Weight of the silk gland on the side operated on the fourth spiracle and that of the gland on the opposite side.

No. of individuals		Weight of silk glands mg.	%	Total weight mg.	Middle division mg.	%	Posterior division mg.	%
1	norm.	97.7	64.6	151.3	78.0	79.9	19.7	20.1
	oper.	53.6	35.4		36.6	68.2	17.0	31.8
2	norm.	46.5	55.9	83.2	34.8	75.0	11.7	25.0
	oper.	36.7	44.1		25.2	68.6	11.5	31.4
3	norm.	42.0	60.9	69.0	27.5	65.5	14.5	34.5
	oper.	27.0	39.1		14.5	53.7	12.5	46.3
4	norm.	36.7	62.6	58.6	26.2	71.3	10.5	28.7
	oper.	21.9	37.4		13.7	62.5	8.2	37.5
5	norm.	29.0	57.9	50.1	20.0	69.0	9.0	31.0
	oper.	21.1	42.1		12.8	60.8	8.3	39.2
6	norm.	31.2	68.0	45.9	19.7	63.0	11.5	37.0
	oper.	14.7	32.0		6.0	40.8	8.7	59.2
7	norm.	24.0	59.3	40.5	13.5	56.3	10.5	43.7
	oper.	16.5	40.7		7.0	42.4	9.5	57.6
8	norm.	22.3	59.3	37.6	11.0	49.3	11.3	50.7
	oper.	15.3	40.7		6.5	42.5	8.8	57.5

In the larvae operated on the sixth spiracle on one side, no evident influence could be found on the size and weight of the silk glands on the operated and unoperated sides. But the percentage of the weight of the posterior division to the total weight of the gland on the operated side was always a little smaller than that on the unoperated side.

According to the description given above, it seems clear that the operation on the fourth spiracle chiefly affects the middle division of the gland, while the operation on the sixth exercises an influence on the posterior division, though very little in grade.

This similarity is also true of the chemical analysis of the cocoons which were spun by the normal and operated worms, as in Table IV.

TABLE IV.

Cocoons spun by the normal and operated worms.

Spiracles operated on both sides	No. of cases	Weight of cocoons (dry matter) mg.			% of sericin		
		max.	min.	mean	max.	min.	mean
Not operated	20	262.0	198.2	229.1	28.3	22.7	23.64
4th	16	229.5	164.5	200.9	24.5	16.5	21.26
6th	7	250.0	189.0	219.9	30.7	24.7	26.97

Unexpectedly, observations of the middle division of the gland on the side operated show no evident difference especially in vacuolation, except the smaller diameter of the gland.

3. THE TESTIS.

In the mature larvae and pupae the testes on the side operated on the second day of the fifth larval stage were always smaller than the normal ones and were sometimes much distorted. However, this state of things was not so conspicuous as in the case of the ovary. The testes on the side operated or not could be easily distinguished by a remarkable difference in the number of pyrene spermatozoa, leaving the apyrene ones intact, as is shown in Table V. Moreover, in the testis on the side operated numerous degenerated forms and abnormal types of spermatozoa were found, differing from that found in the normal. But in the rate of fertilization no difference could be seen between the moth normal and that emerged from the individual operated on both sides. Consequently it seems clear that in the testis of the moth under operation the number of spermatozoa is quite enough to fertilize the eggs of one or two females, though much less than in that of the normal one.

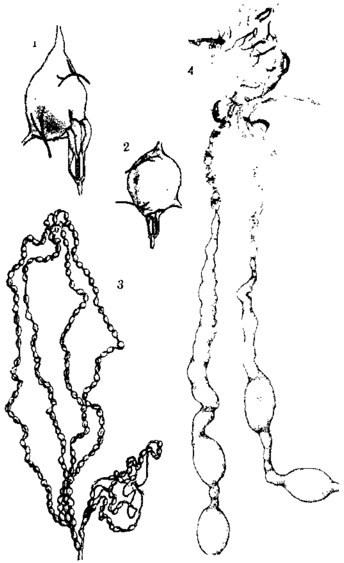
TABLE V.

Number of the bundles of pyrene and apyrene spermatozoa in the testis of the operated and unoperated sides.

Pupal stage		Size of testis (mm.)	Follicle I		Follicle II		Follicle III		Follicle IV		Total	
			pyr.	apyr.	pyr.	apyr.	pyr.	apyr.	pyr.	apyr.	pyr.	apyr.
1st day	norm.	34.0×15.6	148	46	138	37	187	48	159	45	632(55.4%)	176
	oper.	27.0×19.9	238	85	224	82	32	34	15	30	509(44.6%)	231
2nd day	norm.	39.4×16.5	373	95	399	99	363	97	364	88	1499(53.3%)	379
	oper.	37.6×19.6	329	76	335	71	312	91	335	75	1311(46.7%)	313

4. The Ovary.

In the mature larvae the ovary on the side operated on the second day of the fifth stage was a great deal smaller than that on the opposite unoperated side. The common sheath of the former exhibits a broken appearance on the second day of pupation (Text-fig. 2), while that of the latter is found to be broken considerably, causing the egg tubes to be exposed (Text-fig. 1).



Text-fig. 1. *Ovary on the third day of pupation on the unoperated side.*

Text-fig. 2. Ovary on the third day of pupation on the operated side.

Text-fig. 3. *Ovaries of the moth operated on one side.*

Text-fig. 4. Terminal portion of ovary on the operated side.

As may be evident from Table VI, the mature larvae and early pupae have the ovary in which the egg cells are much less in number on the operated than on the unoperated side. This is also true of the adult stage, as in Table VII and Text-fig. 3.

The same result was obtained on the ovaries of the worms which were operated in the more later stage, that is, on the day before maturation, as is shown in Table VIII. The ovaries of the larvae operated at the mature stage could not be studied, because unfortunately the operation at that time affected the worm-body so seriously that all of the worms were killed.

Further, mention will be made in some detail of

the cases of Nos. 1 and 2 (Daizo) in Table VII. In these individuals the egg tubes of the ovaries on the operated side are much shorter than those on the opposite unoperated side and contain a fairly large number of incomplete eggs in addition to some complete ones (Table IX). There can be distinguished the portions containing developed and undeveloped eggs, the latter gradually becoming smaller, as in Text-fig. 4.

TABLE VI.

Number of egg cells in the ovaries of the operated and unoperated sides.

Stages		Normal	Operated
Mature larvae	Size of ovary	18.5×9.2 (mm.)	14.5×11.0 (mm.)
	Number of egg cells	519 (58.4%)	370 (41.6%)
2nd day of pupation	Size of ovary	19.5×15.4 (mm.)	16.0×11.8 (mm.)
	Number of egg cells	1016 (54.0%)	865 (46.0%)

TABLE VII.

Number of eggs in the ovaries on the operated and unoperated sides of the moths, which were operated on the second day of the fifth larval stage.

Race	No. of individuals	Norm.	Oper.	Total	Norm. (%)	Oper. (%)	Rate of asymmetry (%)	Remark
Daizo	13 individuals	Control		max. 397 min. 283 mean 329	Control		max. 4.14 min. 0.15 mean 1.75	
	1	166	44	210	79.0	21.0	58.0	
	2	101	48	149	67.8	32.2	35.6	
	3	119	99	218	54.6	45.4	9.2	
	4	147	129	276	53.3	46.7	6.6	—
	5	174	157	331	52.6	47.4	5.2	
Sekigi	20 individuals	Control		495 406 460	Control		6.86 0.00 1.79	
	1	195	93	288	67.7	32.3	35.4	Operated completely
	2	178	125	303	58.7	41.3	17.4	
	3	173	129	302	57.3	42.7	14.6	
	4	214	164	378	56.6	43.4	13.2	Operated incompletely
	5	249	193	442	56.3	43.7	12.6	
	6	285	321	516	55.2	44.8	10.4	
	7	198	169	367	54.0	46.0	8.0	

Table VIII.

Number of eggs in the ovaries on the operated and unoperated sides of the moths, which were operated on the day before maturation.

Race	No. of individuals	Norm.	Oper.	Total	Norm. (%)	Oper. (%)	Rate of asymmetry (%)	Remark
Sekigi	1	221	97	318	69.5	30.5	39.0	Operated completely
	2	135	70	205	65.9	34.1	31.8	
	3	196	127	323	60.7	39.3	21.4	
	4	260	172	432	60.2	39.8	20.4	
	5	204	167	371	55.0	45.0	10.0	Operated incompletely
	6	167	138	305	54.8	45.2	9.6	
	7	225	191	416	54.1	45.9	8.2	

Table IX.

Length of egg tubes and number of eggs in the ovaries on the operated and unoperated sides.

No. of individuals			Normal				Operated			
1	Mean value of egg size		Length × Breadth = 103.4				Length × Breadth = 100.4			
	Developed	Length of egg tubes	1	2	3	4	1	2	3	4
			61.0	60.0	62.5	58.7	13.0	22.1	17.0	19.0
		Number of eggs	166				44			
			43	43	42	38	9	16	8	11
	Undeveloped	Length of egg tubes	—	—	—	—	10.5	4.6	7.0	5.0
		Number of eggs	11				49			
							17	9	15	8
	Total length of egg tubes		61.0	60.0	62.5	58.7	23.5	26.7	24.0	24.0
2	Mean value of egg size		Length × Breaeth = 92.8				Length × Breadth = 89.0			
	Developed	Length of egg tubes	75.0	70.6	71.0	64.6	21.8	21.7	13.9	11.2
		Number of eggs	101				48			
							16	14	10	8
	Undeveloped	Length of egg tubes	—	—	—	—	12.9	10.5	13.2	13.0
		Number of eggs	9				46			
							13	9	14	10
	Total length of egg tubes		75.0	70.6	71.0	64.6	34.7	32.2	27.1	24.2

The eggs developed in the ovary on the operated side, though presenting no difference in their function, are a little smaller than those on the unoperated side.

In order to examine definitely the hatching ability of the eggs on the operated side, experiments were made with the eggs laid by the moths which were operated on both sides at the larval stage. All the eggs hatched out nearly at the same time within the period as in the normal case.

Conclusion.

So far as the preliminary investigation is concerned, a positive correlation can be observed between the size of the fourth spiracle and the weight of the silk gland, as well as between the sizes of the sixth spiracle and the sexual organ. With regard to this I am inclined to think that it is secondarily induced by the correlation which may exist between the size of the spiracle and the development of the trachea. In fact the experiments here carried out appear to stand in favour of this supposition.

The operation made on the sixth spiracle, though of a slight degree, exercises an influence on the posterior division of the silk gland and results in the slight reduction of fibroin; while that made on the fourth spiracle greatly affects the middle and posterior divisions and also causes the reduction of sericin and fibroin. Consequently it is self-evident that the trachea of the fourth spiracle plays a more important rôle in the development of the whole silk gland, or in the production of silk substances than any other trachea. This is entirely in coincidence with that obtained from the preliminary investigation, in which a correlation was traced between the weight of the silk gland and the size of the fourth spiracle.

The silk gland which was affected by operation made on the fourth spiracle presents an aspect quite similar in the character and quantity of vacuoles to that not affected. Consequently it may be concluded that there is no direct relation between the vacuole and the trachea in the middle division. So far as observations go, the trachea scarcely has the direct relation to the function of the gland, though the former plays an important rôle in the development and settlement of the latter.

In his paper Dr. MACHIDA placed on record the decrease of

spermatozoa and the appearance of abnormal spermatozoa in the transplanted testis of the silkworm, without proposing any physiological explanation. This similarity is also true of the testis on the operated side*). In these two cases I think it advisable to ascribe the most reliable cause to respiratory injury, or to the amputation of tracheae which are distributed on the testis (in the case of Dr. MACHIDA) and *to the operation on the tracheae (in my case).*

In the ovary influenced by respiratory injury there occurs the decrease of the germ cells and therefore of the mature ova. The *decrease of the latter is much more conspicuous as compared with* that of the spermatozoa in the testis affected by operation. The decrease of the mature eggs amounts to more than 30%, while that of the egg cells is about 10%, much as in the case of spermatozoa.

The phenomenon just mentioned may be explained by a consideration of the following facts. In the testis the tracheae enter the testicular follicles, while in the ovary they extend to the peritoneal sheath, without invading the content of the egg tubes. Moreover, the testis is enclosed in the common sheath throughout the life, while the ovary comes to lie in the body cavity on the third or the fourth day of pupation. Therefore the influence of operation on the testis appears to be more remarkable as compared with the case of ovary. But the reverse is true. When the egg tubes become exposed to the body cavity, the tracheae which are distributed in the sheath are necessarily altogether broken. This appears to me to result in the hindrance of the development of the egg tubes, on account of the entire absence of the remarkable development of the tracheae, differing from the condition found in the normal case.

In this way, the insufficient function of the trachea appears to result in the remarkable reduction of the mature ova at the imaginal and the late pupal stage, at which, in addition to a few mature ova, a large number of immature ova are found at the terminal portion of the egg tube.

This explanation, I think, is also applicable to the adipose moth, in which a relatively small number of eggs can be found, because of the reduction in number of the mature ova, not only due to direct

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*) Dr. MACHIDA was kind enough to inspect my preparation. The abnormal spermatozoa, though he did not regard them to be entirely of the same nature with those in his own investigation, appear to me to be atypical.

injury upon the ovary but also to respiratory injury caused by the pressure of the fatty mass on the trachea which extends to the egg tube.

Judging from the results obtained by the present investigation, it is self-evident that the maturation of the ova is apt to be greatly influenced at the late pupal stage by the environmental and physiological conditions.

Notwithstanding their remarkable reduction in number and their smaller size, the eggs influenced by operation do not differ from the normal ones in any physiological condition. This result well coincides with that obtained by UMEYA in his experiments on the ovarian transplantation between different breeds of the silkworm. In any case of the transplantation experiments successfully carried out, the reduction in number of eggs was inevitable, and those eggs made the individuals far more vigorous than the control. To my mind, the cause of this reduction of the eggs appears to be due to respiratory injury caused by the amputation of the trachea on the operation of transplantation, as in the case of the testis.

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Supplementary Study of the Morphogenesis of the Thyreoid Gland in the Chick (Gallus domesticus).

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With twenty Figures in the Text.

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Introduction.

With regard to the development of the thyreoid gland in the chick investigation has hitherto been made by some authorities such as MÜLLER, MALL, KASTSCHENKO, VERDUN and FOSTER-BALFOUR. However, a number of questions have been still left unsettled. In order to elucidate some of those problems and to confirm to what extent NORRIS' classification of the morphogenesis of the human thyreoid gland can be applied to the case of the chick[1], the present work has

[1] While this work was going on, BRADWAY published a valuable paper on the morphogenesis of the thyreoid follicles in the chick, in which he briefly dealt with the classification.

been undertaken at the suggestion of Professor T. KABURAKI, to whom I wish to tender my sincere thanks for advice and criticism, as well as for providing me with accommodation in his laboratory. My thanks are also due to Dr. Y. TAMURA for kind advice rendered me at the beginning of this work.

Material and Methods.

The eggs, upon which the present work is based, are chiefly of the white leghorn, cochin and their hybrid, and were kept at temperatures of 38–40°C. in the incubation apparatus.

The material was fixed with best results by BOUIN'S solution in the early stage of development and by formalin in the later stage. For staining the material in toto neutral borax carmin was used. After dehydration in 90% and absolute alcohol the material was transfered to cederoil xylol which was successively replaced by xylol and xylol paraffin. Thence, it was embedded in paraffin at 52–58°C.

The sections, almost always 10μ in thickness, were made in three directions, i. e., sagittal, frontal and transverse, in which sagittal sections are suitable for the study of transition of the gland, frontal sections for the determination of the relation of the gland to the aortic arches and other pharyngeal derivatives, and the last transverse sections were for special purposes.

Haematoxylin and eosin were often used for staining.

Observations.

NON-PAIRED STAGE.

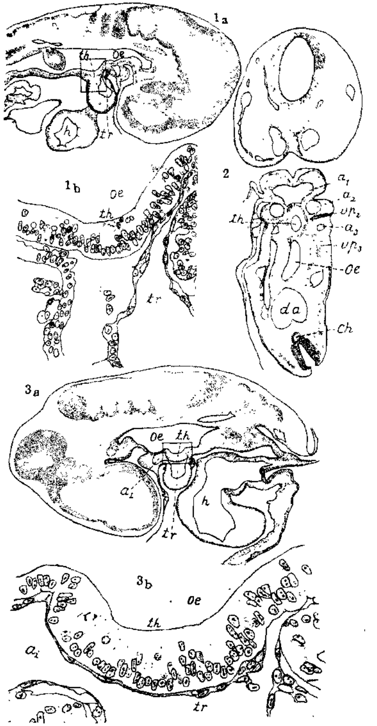
ORGANOGENESIS.—The rudiment of the thyreoid gland is found already at the stage of 1.75 days in incubation arising as a shallow evagination of the floor of the pharynx at the level of the second visceral pouch. This evagination occupies a comparatively extensive region, its wall being twice as thick as the general pharyngeal wall (Text-fig. 1 a & b). At the stage of two days it deepens slightly and assumes a shape ovoidal in frontal section. Ventrally it comes in close contact with the roots of the second and third aortic arches, of which the latter do not yet develop into large vessels (Text-fig. 2).

At the stage of 2.25 days the gland wall attains a considerable thickness and is easily distinguished from the neighbouring pharyn-

geal wall (Text-fig. 3 a & b). Its evagination gradually proceeds to the formation of a distinct central lumen, and it tends to be suspended in the ventral aorta which develops into a sinus-like body. At about this stage the fourth aortic arch makes its appearance.

At the stage of 2.5 days (Text-fig. 4) the evagination bends backwards along the ventral aorta and assumes a keel-like appearance, much as in the case of the embryo, 14 mm. long, of *Squalus acanthia* (NORRIS). Six hours after this stage this keel-shaped evagination is transformed into a spherical body, the ventral third of which is in close contact with the ventral aorta (Text-fig. 5).

The communication of the lumen of the gland with the pharyngeal coelom, though still traced in an embryo of 2 days and 22 hours in incubation, is in general nearly closed at the stage of



Text-fig. 1. Sagittal sections through the head region of the 1.75 day-old embryo (a) and showing the structure of the thyreoid anlage (b).

Text-fig. 2. A frontal section through the pharyngeal region of the 2 day-old embryo.

Text-fig. 3. Sagittal sections through the head region of the 2.25 day-old embryo (a) and showing the structure of the thyreoid anlage (b).

a_1-a_3 aortic arches from the first to the third, *ch* notochord, *d. a.* doral aorta, *h* heart, *oe* oesophagus, *th* thyreoid gland, *tr* trancus arteriosus, vp_2, vp_3 second and third visceral pouches.

Text-fig. 4. A sagittal section showing the keel-shaped thyreoid gland in the 2.5 y-old embryo.
Text-fig. 5. Ditto, showing the spherical thyreoid gland at the 2.75 day-old embryo.
Text-fig. 6. A sagittal section through the thyreoid gland of the 3 day-old embryo.
Text-fig. 7. A sagittal section through the thyreoid gland of the 3.5 day-old embryo.
Text-fig. 8. A sagittal section through the thyreoid gland of the 3.75 day-old embryo.
a ventral aorta. Other letters as in Text-figs. 1–3.

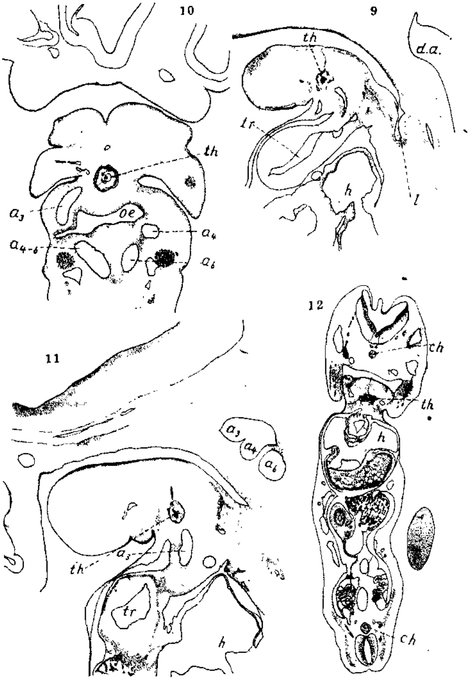
3 days and is represented by a thyreoglossal duct (Text-fig. 6). At this stage the gland wall attains a remarkable thickness. In spite of the highest development of the third and fourth aortic arches, the second arch begins to degenerate. With this degeneration the ventral aorta which is located anterior to the third aortic root is considerably reduced, and the spherical thyreoid gland with its central lumen shows a tendency to elongate antero-ventrally. At the same time the gland becomes gradually separated from the aorta by the invasion of mesenchyme.

In the 3.5 day-old embryo the gland, which was of a spherical shape in the preceding stage, is found to flatten as a whole, especially in the posterior region (Text-fig. 7). It is still placed between the second gill clefts and near the bifurcation point of the second arch. From this stage onwards, the thyreoglossal duct begins to lengthen in association with the antero-ventral elongation of the gland (Text-fig. 8). In the 3.75 day-old embryo the foramen caecum is found to develop considerably.

At the stage of 4 days and 3 hours (Text-figs. 9 & 10), the gland assumes a shape polyhedral in frontal section. Its long axis is in the same direction with the thyreoglossal duct which fairly lengthens and is perpendicular to the pharyngeal wall. The gland presents a concavity at the postero-ventral aspect or at the anterior angle of the aortic root. The central lumen is found to be located near the connection of the thyreoglossal duct with the gland.

From this stage onwards, the duct exhibits further elongation. At the stage of 4.25 days (Text-figs. 11 & 12) it is found extremely narrowed and curved forwards. The gland is transformed into an elliptical shape in sagittal section and occupies a position in the prolonged line of the curved duct just mentioned. On the other hand, the gland is observed in frontal section with the lateral elongation, which comes in contact with the third arches on both sides. This elliptical shape does not persist for a long time and is transformed into a triangular body swollen outwards, which keeps its ventral portion in close contact with the third arch.

So far as observations go, the thyreoglossal duct presents no trace of lumen throughout and is partly broken just before the pairing of the gland sets in. It seems to be altogether atrophied at the stage between 4.25 and 4.5 days. Its disappearance may be induced either by the first break near the gland or by the uniform thinning.



Text-fig. 9. A sagittal section through the thyreoid gland at the stage of 4 ys and 3 hours.

Text-fig. 10. A frontal section of the same.

Text-fig. 11. A sagittal section through the thyreoid gland at the stage of 25 days.

Text-fig. 12. A transverse section through the thyreoid gland at the stage of 4.25 ys and 1 hour.

, a_6 fourth and sixth aortic arches, *l* lung. Other letters as in Text-figs. 1–3.

Occasionally a solid cell mass is found protruded into the pharyngeal coelom at the point of the foramen caecum. Shortly after, it disappears altogether.

Histogenesis.—At the stage of 1.75 days the rudiment of the thyreoid gland which arises as an evagination of the pharyngeal wall is made up of high columnar cells, the nuclei of which, though at first elliptical, assume a spherical shape and are arranged near the distal end (Text-figs. 1b & 3b). As a result there can be distinguished *two zones, an outer nucleate and an inner cytoplasmic, the latter* presenting some mitotic figures. The cell boundary is fairly distinctly recognized in the cytoplasmic zone. These two zones persist for a fairly long time up to about the stage of 3.5 days. As the result of the rapid cell-proliferation in the outer nucleate zone, however, the newly produced cells migrate towards the inner cytoplasmic zone, accompanying with the decrease in number of the nuclei in the outer zone. Thus, the gland makes a rapid development.

Irrespective of the rapid growth of the outer nucleate zone, the nuclei are much fewer in the dorso-posterior portion near the thyreoglossal duct than in the other portion of the gland. This is true of the 2.5 day-old embryo with the keel-shaped gland, as well as of the 3 day-old embryo with the spherical gland.

In the 3.5 day-old embryo the gland wall is considerably thickened and has the nuclei which proliferate at the periphery and gradually migrate towards the inner cytoplasmic zone. At the more advanced stage the rapid cell-proliferation and the cell-migration result in the growth of the gland chiefly at the antero-ventral aspect. The central lumen, though keeping its proper position, is highly reduced by the thickening of the wall. The cell-migration is also observed at the stage of 4 days and 3 hours in the lateral thickened part of the gland, which is of a polyhedral shape in frontal section (Text-fig. 10).

One peculiarity may be attributable to the fact overlooked hitherto by many observers:—after the closure of the communication of the gland with the pharyngeal coelom, the cytoplasmic mass which contains 4 or 5 nuclei can be seen in the central lumen. These nuclei are a little smaller and are stainable more deeply than the proper thyreoid nuclei and become deformed. This cytoplasmic mass is found to persist still at the stage of 4 days and 3 hours. Judging from its close contact with the inner migrated nuclei in the laterally thickened wall, it appears to be produced by the overgrowth of the

thyreoid nuclei (Text-fig. 10). Following the cell-migration the *secondary lumina make their appearance as follows:*

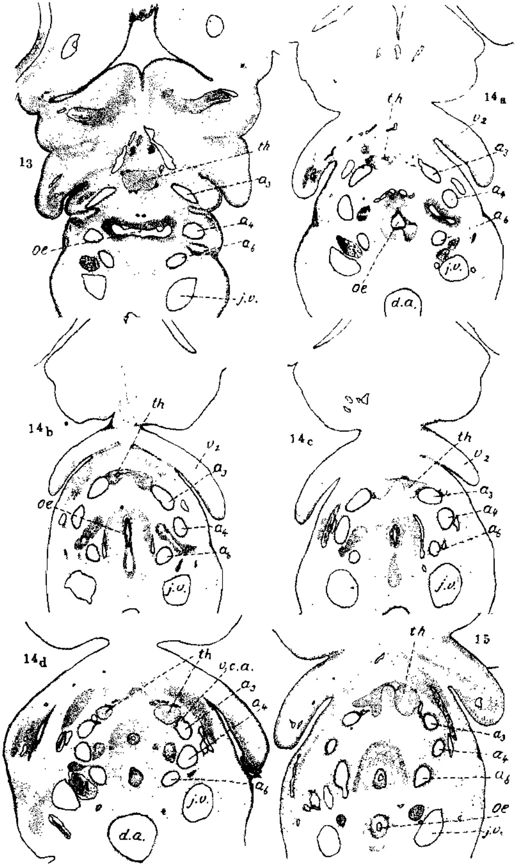
As the development goes on, almost all of the nuclei migrate towards the inner side, remaining a unicellular layer at the periphery. *Thus, the middle cytoplasmic zone appears between the outer and* inner nucleate zones. After the stage of 4 days the thyreoid gland elongates laterally and exhibits cytoplasmic islands here and there by *the rapid increase of the nuclei. In these islands the secondary* lumina make their appearance by the rearrangement of the cells. They are characterized, as in the case of the central lumen, by the *presence of a clear cytoplasmic zone. These lumina, though very* small in size, are distinctly observed. Occasionally the lumen is so small that we can scarcely demonstrate it, exclusive of the cytoplasmic zone. The secondary lumina are seen especially in such parts where the development rapidly proceeds as in the laterally elongated part, the antero-ventrally developed part, etc. However, the duration of the occurrence of these lumina is less than six hours. As the cell-proliferation goes on, the lumina disappear to make the gland homogeneous, but the central lumen, though greatly reduced, persists in the upper portion of the gland near the thyreoglossal duct.

Pairing Stage.

In order to elucidate the mechanism of pairing of the gland, a large series of embryos, which were of the same race and were incubated at the same time, were taken at intervals of one hour from the stage of 4.25 days and 1 hour to that of 4.75 days and 1 hour. So far as the examination showed, the pairing takes place chiefly at two different periods, one at the stage of 4.25 days and 1 hour, the other at the stage of 4.5 days and 2 hours. In these two cases the time required for the pairing is less than 6 hours. On one occasion I found a splendid series of examples in which the pairing was accomplished within 4 hours, beginning from the stage of 4.25 days and 1 hour (Text-figs. 13 & 14 a, b, c, d).

The pairing takes place thus:—after the conversion of the thyreoid gland into the homogeneous solid body which elongates laterally *between the third aortic arches and has the lumen near its centre,* the gland causes a depression at the mid-caudal aspect.

As this depression proceeds cephalads the gland becomes constricted at the middle and finally separates keeping both sides in close contact



Text-fig. 13. A frontal section through the thyreoid gland at the stage of 4.25 days and 1/2 hour.

Text-fig. 14. Frontal sections through the thyreoid gland at the stages of 4.25 day+1 hour (a), 4.25 days+2 hours (b), 4.25 days+4 hours (c) and 4.25 days+5 hours (d).

Text-fig. 15. A frontal section showing the horse-shoe shaped thyreoid gland.

j. v jugular vein, v_2 second visceral arch, *v. c. a* ventral carotid artery. Other letters as in Text-figs. 1, 2, 3, & 10.

with the third aortic arches. In some cases it assumes a horse-shoe or dumb-bell-like shape (Text-fig. 15).

To my mind, the pairing of the gland appears to take place by a phylogenetic cause, because of the absence of any aggregated cell-mass which induces the bending of the gland.

After completion of the pairing, each gland is found as a solid body lying near the third aortic arch. The central lumen, though transmitted into either of the paired glands, soon loses the clear cytoplasmic zone arround it and is reduced to obliteration without playing any rôle in the later morphogenesis.

Let us now pass on to the observation of the vascular system which stands in close relation to the thyreoid gland. In spite of its close contact with the ventral aorta at the bifurcation point of the second aortic arch until the stage of 3 days, the gland soon becomes separated from the ventral aorta by the invasion of the mesenchyme. At about the 4 day-old stage it is found to lie between the second and third aortic roots, above the level of the ventral aorta which remains merely as a feeble blood vessel. When inspecting the sections, numerous capillaries or vascular sinusoids are found around the gland. A close examination reveals the fact that two arterioles are found to develop from the third aortic arch near the branching part of the ventral aorta and tend to surround the gland. At about the 4.5 day-old stage a pair of well-developed ventral carotid arteries[2] are found running along the latero-dorsal side of the gland, extending to near the mandibular region. The gland which is thus surrounded by the sinusoids makes a steady development as a homogeneous solid body up to the 5.25 day-old stage.

PAIRED STAGE.

CAVITY FORMATION STAGE.—At about the stage of 5.25 days the gland passes on to the cavity formation stage which was first mentioned by NORRIS in the elasmobranchs and man. This stage appears to last merely for a few hours (from 5.25 to 5.5 days) and is easily overlooked. In the gland, at certain distances from its border, are found some cavities which are isolated from one another and are surrounded by round thyreoid nuclei (Text-fig. 16 a, b). They may

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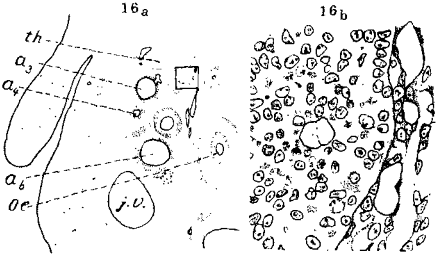
[2] In spite of the work of TWINNING the ventral carotid artery has generally been misinterpreted as the external carotid artery. This artery becomes atrophied in the later stage.

be considered to be caused by the fusion of vacuoles which appear in the gland. Though difficult to determine, they appear to be intracellular, because of the occasional presence in the cavities of the remnants of cell boundaries between the fused vacuoles.

Here it might be noticed that this cavity is entirely different from the secondary cavity or the central lumen already mentioned.



Text-fig. 16a. A section of the 5.25 day-old embryo, showing the cavity formation stage of the gland.

Text-fig. 16b. Ditto, showing the structure of a part of the gland. Index letters as in Text-figs. 1, 2, 3, 10 & 14.

VASCULAR STAGE.—At the 5.5 day-old stage the gland becomes exceedingly lobulated (Text-fig. 17). The capillaries which arise from the sinusoids around the gland are found to invade the body, in company with the lobe formation. In general there cannot be seen any closed cavity. If present, the cavity is very scarce. In the capillaries branching off in the gland are found erythrocytes here and there; consequently it may be said that the capillaries are of the blood vessel. As to whether these capillaries are of the arteriole or venule, I am not in a position to make any assertion. On the other hand, around the gland body occur two arteries which are given off from the third aortic arch. Of these one arises from the third arch which occurs close to the gland and directs towards the base of the gland.

The other arises from the same arch at a point a little lateral to the former, and runs along the latero-anterior angle of the gland.

Beyond doubt the former invades the gland. The latter appears to give off a branch at a point near to the origin of the subclavian artery to invade the gland.

Text-fig. 17. A transverse section (a little inclined) through the thyreoid gland of the 5.5 day-old embryo, showing vascularity to a certain extent.

Text-fig. 18. A sagittal section through the thyreoid gland at the stage of 6 days and 3 hours.

Text-fig. 19. A sagittal section through the left thyreoid gland in the 7 day-old embryo.

Text-fig. 20. A sagittal section through the left thyreoid gland in the 8 day-old embryo. *l. c. d.* left cuvierian duct, *s. a.* subclavian artery, *v. n.* vagus nerve. Other letters as in Text-figs. 1, 2, 3 & 10.

Here we necessarily face the following questions:

1) Whether the two arteries which are given off from the third arch are of the same nature with the sinusoids around the gland.

2) Whether the arteries and sinusoids are independently developed and anastomose secondarily with each other.

3) Whether the sinusoids are independently developed as of a venous nature, and whether the arteries also independently invade the gland as of an arterial nature.

With regard to these questions I am not in a position to give any definite solution, and the questions deserve future investigation[3].

In company with the blood vessels a negligible amount of the mesenchyme invades the gland, differing from that found in the human thyreoid.

Up to the appearance of the thyreoid follicles the gland is perforated by the blood vessels and finally attains such a condition as it is chiefly composed of anastomosing cell-cords (Text-figs. 18–20).

In sections the anastomosing cell-cords are found to consist of about two cells in thickeness. Here I was unable to determine whether the thyreoid is made up of fenestrate bands or plates as mentioned by NORRIS in the human thyreoid.

Even at the later vascular stage the cavity formation still goes on. In an embryo of 10 days and 5 hours are found two large cystic and spherical cavities in the gland. I think it advisable to regard this state of things as a special result of vacuole formation.

At the 11 day-old stage the gland is found to assume the highest vascular appearance.

As regards the relation between the gland and the jugular vein:—At the 7 day-old stage the vein is found to approach fairly close to the gland, whilst the common carotid artery is gradually separated from the gland. At the 9 day-old stage the broad and free vascular connection can be seen between them. At the same time the arterial branch given off from the common carotid artery is found to make its way into the thyreoid blood sinus.

FOLLICULAR STAGE.—At the stage of 11 days and 3 hours the gland presents the highest degree of vascularity, and a number of small

3) At a stage of 5.5 days the sinusoid is found to connect with the arteriole which is given off from the vessel at a point near to the origin of the subclavian artery. Therefore, it may be said that the sinusoid is of the same nature with the artery.

vacuoles make their appearance here and there in the cell-cords. However, these vacuoles stand at variance with the non-follicular cavities at the cavity formation stage. At the stage of 11 days and 12 hours the vacuoles considerably increase in size and number and transform the gland into a colony of vacuoles. As development goes on, the vacuoles enlarge independently and fuse with one another. The thyreoid nuclei become arranged so as to surround the vacuoles. The vacuoles are of a chromophobe character, as mentioned by BRADWAY.

At the 12 and 13 day-old stages, the gland is found to consist of a number of series of beads, which contain the chromophile liquid or colloid, surrounded by the thyreoid nuclei. This chromophile colloid is thought to be derived from the chromophobe colloid and represents the rudiment of the later thyreoid follicle. On some occasions the content of this chromophile colloid is found to be discharged into the blood sinus, attaching to the follicular wall. At the 14 day-old stage a remarkable increase of beads arranged in series is demonstrated.

From the 15 day-old stage onwards, the thyreoid follicles gradually increase in size and number and tend to lie in close contact with one another, obliterating the blood sinus between them. In this way the gland assumes a feature almost similar to that found in the newly hatched chick.

At about the 17 day-old stage the cell-proliferation takes place here and there, and the secondary follicles make their appearance. Thus, the formation of the thyreoid follicles goes on.

Here it must be noted that in the epithelial cells which form the follicular wall clear vacuoles are found near the inner margin. Occasionally these vacuoles open out freely into the central follicular lumen.

Discussion.

NORRIS published an important paper on the morphogenesis of the human thyreoid gland, classifying it according to a new system in the following manner:

1. Pre-anlage stage,
2. Early anlage stage,
3. Early growth stage,
4. Beginning separation stage,
5. Complete separation stage,
6. Cavity formation stage,
7. Plate stage,
8. Follicular stage.

So far as the morphogenesis of the thyreoid gland in the chick is

concerned, however, I think it better to classify the developmental processes according to the following system:

I. Non-paired stage,
1. Anlage stage,
2. Early growth stage,
II. Pairing stage,
III. Paired stage,
1. Cavity formation stage,
2. Vascular stage,
3. Follicular stage.

In this system the anlage stage includes the pre-anlage and early anlage stages proposed by NORRIS. The early growth stage extends from the appearance of the gland of a keel-like shape to the disappearance of the thyreoglossal duct; consequently this stage may be said to correspond to NORRIS' beginning and complete separation stages in combination. The vascular stage is proposed for the plate stage of NORRIS, on account of the presentation of the highest vascularity.

With regard to the first appearance of the thyreoid rudiment there is a difference of opinion. According to the observations conducted hitherto the anlage is said to appear for the most cases, extending from the beginning of the second day to that of the fourth day of incubation. So far as my observations went, it made its appearance already at about the 1.75 day-old stage. This entirely concurs with that determined by LILLIE. According to him, the rudiment appears at the stage of 12 somites which corresponds to that of 1.75 days.

The appearance of the thyreoid rudiment as an evagination shows that the chick is of a type different from the elasmobranchs and man, in which the anlage protrudes into the pharyngeal lumen as a solid budding. *As the evagination goes on, the gland turns backwards to* assume a keel-like shape, much as in the elasmobranchs, dipnoi and salamander. This state of things may be regarded as of some phylogenetic nature.

Some interest is attached to the antero-ventral elongation of the gland, which does not take place in the lower vertebrates. Perhaps this may be partly caused by the special position of the aortic arches. On this point, however, I am not in a position to make any assertion.

No trace of such a bilobation as reported by NORRIS can be seen in the thyreoid rudiment of the chick.

The central lumen, though not found in the human thyreoid, can be clearly traced in the case of the chick even after the completion of

In the thyreoid of the salamander are demonstrated by MIZOGUCHI such secondary lumina as are observed in that of the chick. These he is inclined to regard as produced by the division of the central lumen. Judging from the differentiation of the inner cytoplasmic and outer nucleate zones and the migration of the thyreoid nuclei from the latter towards the former, however, these lumina appear to be produced independently of the central lumen.

The thyreoglossal duct is produced as a stalk solid but not hollow. So far as observations go, the disappearance of the stalk is not so complex as mentioned by NORRIS and may be induced either by the first break near the gland or by the uniform thinning.

As to the period when the separation and pairing of the gland take place, FOSTER and BALFOUR set forth that by the fifth day the gland ceases to be connected with the epithelium of the throat and becomes at the same time bilobed and by the seventh day it travels somewhat backwards, and the two lobes are completely separated from each other. From a consideration of some figures given by VERDUN, LILLIE states that the thyreoid sac (median thyreoid of the author) loses all connections with the pharyngeal epithelium on the fourth day, and on the seventh day it becomes divided into two massive lobes placed laterally. KELLICOTT appears to be of the same opinion. Though no mention is made of this feature, MALL gives an illustration (Pl. II, Fig. 23) of the pairing of the gland, which just corresponds to that of mine. KASTSCHENKO mentions that the gland remains unpaired up to the eighth day of incubation. BRADWAY says that by the end of the fifth day the gland is completely subdivided into two solid bodies, separated from the pharynx. So far as my observations are concerned, the break of the thyreoid stalk and the pairing of the gland are accomplished by the end of the fifth day. At the 5.5 day-old stage the gland is found to be vascular.

The non-follicular cavities surely make their appearance previous to the invasion of the blood vessels, as mentioned by NORRIS. But this stage is very short. There remains some doubt as to whether these cavities are solely of a mechanical nature so as to make the invasion of the blood vessels easy. I should like to interpret them to be of a physiological nature and to secrete something which does not stain. The cavity formation appears to take place independently of the vascular process, though the former process exercises an influence secondarily on the latter.

The chief cause which produces the thyreoid follicle is due to the generation of chromophobe vacuoles which represent the early stage of colloid, as well as to the rearrangement of the thyreoid nuclei which exist near them. The budding or constriction which has been mentioned as one method of follicle proliferation is thought to be the result of the fusion of these colloids.

Summary.

1) The rudiment of the thyreoid gland makes its appearance already at the 1.75 day-old stage as a shallow evagination of the floor of the pharynx at the level of the second visceral pouch. It is made up of high columnar cells arranged in a single layer and presents two zones, the inner cytoplasmic and the outer nucleate.

2) In the 2.5 day-old embryo the gland is of a keel-like shape.

3) In the 2.75 day-old embryo the gland is transformed into a spherical shape.

4) At about the stage of 3 days the communication of the lumen of the gland with the pharyngeal coelom is in general altogether closed.

5) With their multiplication the thyreoid nuclei gradually migrate towards the inner side, to result in the formation of three zones, the inner and outer nucleate and the middle cytoplasmic. In the middle zone some secondary lumina make their appearance here and there.

6) The thyreoglossal duct begins to elongate at the 3.75 day-old stage and becomes atrophied at about the 4.25 day-old stage. This is also the case with the secondary lumina.

7) The pairing of the gland is performed within a few hours, beginning from about the stage of 4.26 days. The central cavity is transmitted into either of the paired glands and is soon altogether atrophied.

8) The paired gland which is of a solid body is followed at about the 5.25 day-old stage by the cavity formation stage. The cavities thus formed are filled up with liquid.

9) At the 5.5 day-old stage the gland begins to assume a vascular appearance. From this to the 11 day-old stage the gland tends to consist of a meshwork of anastomosing cell-cords.

10) At the 11 day-old stage the rudiment of colloid, which is at

first of a chromophobe character and is secreted near the nuclei, makes its appearance.

11) The cell-cords are turned into some series of beads at the 12 day-old stage, at which the colloid becomes exceedingly chromophile.

12) *After the 15 day-old stage the gland assumes an appearance such as is found in the chick just hatched out, but it is very far from the adult condition.*

13) *The proliferation of follicles appears generally to take place in the epithelial cells multiplicated between the follicles.*

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On the Metamorphosis of two Japanese Freshwater Shrimps, Paratya compressa and Leander paucidens, with Reference to the Development of their Appendages.

By

Yu Yokoya.

With Plates VIII–XV and nineteen Figures in the Text.

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Introduction.

While considerable attention has been paid to decapod larvae on the Atlantic coast, not so many studies have been published on the Pacific side. In Japan Prof. K. Kishinouye (18, 19), Mr. K. Nakazawa (22, 23) and Mr. I. Soejima (26) published their studies on the decapod larvae, but these studies are mostly about the larvae of the species belonging to the tribe Penaeidea, Penaeidae and Sergestidae; and only one is about Loricata. On the other hand Mr. H. Aikawa (1, 2, 3) described the larvae of some species of Brachyura. Mr. H. Ichô (16) briefly described the larvae of some stages of two fresh water prawns, *Bithynis nipponensis* (*Palaemon nipponensis*) and *Palaemon paucidens* (*Leander paucidens*):—this is the only study I know on the larval forms of the species belonging to the large group Caridea.

Prof. C. Ishikawa (17), however, reported an interesting fact on the egg

cleavage and on the earlier development of a shrimp, *Paratya compressa*. Afterwards I (31) studied the metamorphosis of the same species and published the results in Japanese as a preliminary report in 1922, but at that time I was unable to rear the larvae in aquaria to the later stages, therefore, collecting the larvae with a surface tow net, I studied them exclusively after the first three stages. Recently I succeeded in rearing the larvae in aquaria and had the opportunity to examine the larvae of this species as well as that of another species *Leander paucidens*; these two species are found abundantly in a small pond in our University ground.

The studies on the larval stages of the decapod crustaceans have been carried on by numerous naturalists in Europe, but most of the studies are confined to a description of the feature of each stage, and are not directed to the transformations from one stage to the next. I want here to describe the transformation as a contribution to the studies on the larval forms as well as to studies of deformations of all the appendages.

The appendages are generally primitive in feature in the early stages, and then gradually deform to the features of the adult. In pursuing the study of this deformation, the distributions of the muscles seem to suggest how some parts of the appendages are transformed from the primitive features to those of the adult.

Method.

The material, on which the present account is based, was obtained from a pond in the University ground; in this pond the present two species of decapod macrura are found. Afterwards I undertook to rear the larvae in aquaria for the purpose of studying the metamorphosis precisely in various ways, but my trials proved the following to be the most convenient and profitable method:—

To rear the larvae in an aquarium I filled it with the water of the pond some days previously and then placed in it the egg-bearing parent animals or some larvae collected from the native pond. In this case putting in the aquarium some species of water weeds such as *Hydrilla verticillata* CASP. var. *Roxburghii* CASP. seems to be attended with good results, but the presence of *Spirogyra* and its allies is evidently harmful. At first I reared the egg-bearing mother animals in the aquarium and obtained the newly hatched larvae; after the hatching out unless the mother animals were removed, the larvae would be much disturbed by them. In my trials of rearing I once obtained a larva of the fourth larval stage by rear-

ing the larvae of the first stage in fresh spring water without food-supply.

On rearing the larvae I examined them two or three times a day under a magnifying lens, and recorded the time when the larvae of a new stage were found. Occasionally larvae of some stages were taken out and precisely observed under the microscope, and then they were preserved in a tube filled with about 80% alcohol for dissecting purposes. On the other hand, by collecting many of the larvae from the native pond and classifying them, I reared one or two larvae in an aquarium, while the others were preserved in spirit as well as those from the aquaria for comparison. Dissecting these materials I examined each appendage under the microscope mostly mounted by water, and by means of the camera the illustrations were made. For the dissecting purposes the alcoholic specimens are more profitable than the living ones. The muscles are noticeable through the transparent crust.

Paratya compressa.[1]

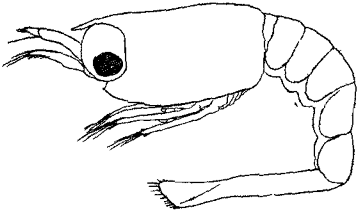
Ephyra compressa DE HAAN 1856 (15); *Miersia compressa*, KINGSLEY 1879; *Xiphocaris compressa*, RATHBUN 1902; *Xiphocaridina compressa*, BOUVIER 1909; *Paratya compressa*, KEMP 1917.

This species is a member of the family Atyidae and is of wide distribution in our country. The form which I have studied appears to be referable to the subspecies *improvisa* established by S. KEMP, though different in some respects from the typical form. It is of small size and measures in the largest specimen 30 mm. long from the base of the rostrum to the end of the telson. The colour of the adult in life varies in different individuals to some extent. Some are light yellowish brown while the others are dark brown. The females, though bearing eggs from March to October, are most fecund in July and August. Some newly hatched larvae were first observed in the beginning of May. The egg is ellipsoid in shape, measuring 0.8 × 0.5 mm. in size. It is at first dark brown in colour, but with the advancement of its development it becomes almost translucent white except the orbital pigment and the yolk granules.

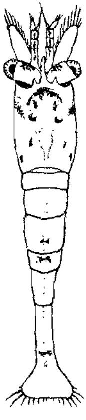
A. METAMORPHOSIS.

I Stage (Text-fig. 1). In the just hatched larvae the body measures about 3 mm. in length from the base of the eye to the end of the telson.

It does not become so transparent as that found in *Leander paucidens*, owing to the presence of chromatophores, yolks, coloured mandible and some other organs. The carapace including the rostrum is nearly as long as the abdomen and is devoid of spinules except one on the antero-lateral corner. The rostrum is slightly bent downwards. The abdomen is dorsally rounded devoid of any trace of spinules, and consists of six segments, of which the last (Pl. VIII, Fig. 1) is very long and its posterior half is laterally expanded to form a thin spatura as usually seen in the larvae of the Caridea.[1] In the anterior half of the last abdominal segment there are two longitudinal muscles, and the posterior margin of the spatular expansion is faintly bilobed bearing seven pairs of plumous setae, of which the outer two pairs lack the exterior series of branching cilia.



Text-fig. 1. *Paratya compressa*, 1st larval stage, viewed from the left side.



Text-fig. 2. *Paratya compressa*, 2nd larval stage, dorsal aspect.

The eyes are of large size. The peduncle of the first antenna is not segmented. In the second antenna the endopodite is represented by a terminally pointed article, which is armed with a long plumous seta and two spinules. Three pairs of the maxillipeds well developed are biramous. Making use of their exopodites, the larvae swim forwards. The anterior two pairs of legs, though not segmented, are distinctly visible while the succeeding pairs are quite rudimentary. The pleopods are not noticeable in this stage. About three days after hatching it passes into the following stage.

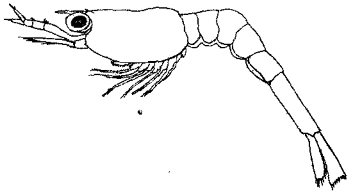
II Stage (Text-fig. 2). The body is closely similar in size and colour to that of the preceding stage so that it is very difficult to distinguish them with the naked eye. The carapace is armed with a pair of the supraorbital spinules. The posterior margin of the last abdominal segment (Pl.

[1] BROOKS & HERRICK (8), GURNEY (10, 11, 13, 14), WILLIAMSON (30) etc.

VIII, Fig. 2) is not bilobed, as in that of the preceding stage, and is almost straight; on this margin there are now found sixteen setae, of which two in the middle are smaller than the others and can not be seen in the preceding stage.

The eyes become somewhat smaller in size. The peduncle of the first antenna is now distinctly three-segmented, presenting a spinule on the inner margin of the proximal segment. The endopodite of the second antenna is provided with three spinules. The anterior three pairs of legs are distinctly segmented, while the remaining two are not segmented. The present stage lasts for three or four days.

III Stage (Text-fig. 3). The body measures about 3.4 mm. long from the base of the rostrum to the end of the telson. The rostrum is produced horizontally. The carapace including the rostrum is a little longer than one half the length of the abdomen. The last abdominal segment is now distinctly divided into two segments, the sixth and the seventh, and the sixth abdominal appendage shows itself. The seventh abdominal segment or the telson (Pl. VIII, Fig. 3) is narrower than that of the preceding stage and is provided with setae on its posterior margin, of which an outer pair is smaller than the others and somewhat advanced in position. In this segment there can be seen two longitudinal muscles. The said sixth abdominal appendage is terminally divided into two branches, and is not distinctly articulated to the basal segment. Of these two branches the outer is provided with six or eight marginal setae, while the inner has two or three minute setae or is wholly devoid of them. This appendage has no trace of muscle.

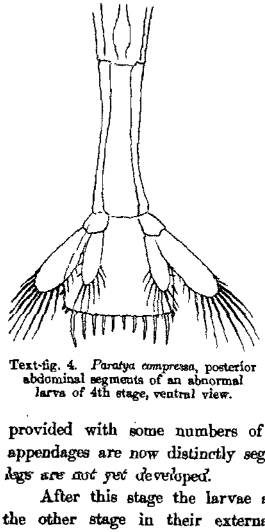


Text-fig. 3. *Paratya compressa*, 3rd larval stage, viewed from the left side.

The outer branch of the first antenna is a little constricted at the middle. The endopodite of the second antenna ends in a rounded extremity with a small seta and is devoid of spinules. The anterior four pairs of legs are segmented, while the fifth is represented by a biramous lobe, but is not segmented. Three or four days after the second ecdysis it enters on the fourth stage.

IV Stage. A single specimen obtained from the aquarium without

food-supply is primitive in feature. The body is about 3 mm. in length, and the lateral margin of the telson is distinctly convex as shown in the Text-fig. 4. The specimens from the pond referable to this stage, however, present an aspect a little more advanced in size and features. The body measures about 3.5–3.8 mm. long, from the base of the rostrum to the end of the telson. The rostrum is a little longer than the eye-stalk. The telson (Pl. VIII, Fig. 4) is trapezoid in shape, with the lateral margins nearly straight and the anterior margin narrower than the posterior. The ratio of the anterior and the posterior margin is about 2 : 3. The outer three pairs of the marginal setae shorten and are spinular in shape, of which two pairs advance in position and pass into the spinules on the lateral margin, while the other lies at the postero-lateral corner. In the sixth abdominal appendage the basal segment is now distinctly articulated with the two branches, of which the inner is provided with some numbers of marginal setae. All the cephalothoracic appendages are now distinctly segmented. The chelae of the anterior two legs are not yet developed.



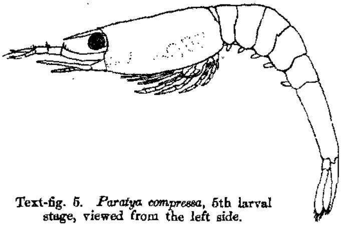
Text-fig. 4. *Paratya compressa*, posterior abdominal segments of an abnormal larva of 4th stage, ventral view.

After this stage the larvae are hardly distinguishable from those of the other stage in their external appearance, so that it is difficult to determine how many days it takes to transform to the next stage.

V Stage (Text-fig. 5). The body excluding the rostrum is about 3.8–4.0 mm. in length. The telson (Pl. VIII, Fig. 5) is somewhat narrower than that of the preceding stage at the posterior extremity, and the anterior end is about three-fourths as wide as the posterior. The endopodite of the second antenna is about as long as, or somewhat longer than, the exopodite or scaphocerite. In the anterior two pairs of pereiopods the penultimate segment is a little dilated inwards so as to form a chela. The abdominal appendages or pleopods appear as bud-like processes.

VI Stage. The body measures 4.0–4.3 mm. long from the base of the rostrum to the end of the telson. The rostrum is elongated and armed

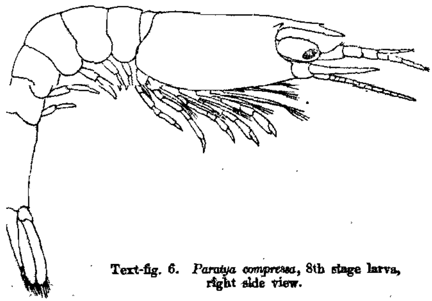
with a tooth on the superior margin. The telson is almost rectangular in shape, decreasing in breadth at the posterior end. In the first antenna the outer flagellum consists of three articles, while the inner has two articles. The flagellum of the second antenna is a little longer than the scaphocerite. The pleopods are now developed and terminally divided into two distinct lobes.



Text-fig. 5. *Paratya compressa*, 5th larval stage, viewed from the left side.

VII Stage. The body without the rostrum is 4.3–4.6 mm. in length. The larva of this stage is alike to that of the preceding stage, but the flagellum of the second antenna is more elongated and the last pair of legs is ordinary in feature for the first time. The telson (Pl. VIII, Fig. 6) is narrower at the posterior end, where it is as wide as or somewhat narrower than the margin at the anterior end. In the pleopods some numbers of short marginal setae appear near the distal end.

VIII Stage (Text-fig. 6). The body without rostrum is about 4.7–5.3 mm. long. The rostrum is about as long as the basal peduncular



Text-fig. 6. *Paratya compressa*, 8th stage larva, right side view.

segment of the first antenna, and is provided with a tooth on the upper margin. In the first antenna the outer branch consists of four articles, while the inner has three articles. The flagellum of the second antenna

exceeds the tip of the scaphocerite by more than one-third of its length. The chelae of the anterior two legs are well developed. In nearly all thoracic appendages the protopodite is a little dilated at the outer margin, and this dilation is a rudiment of the epipodite. The pleopods are provided with marginal setae, but these setae are still short in length.

IX Stage. The length of the body excluding the rostrum is about 5.3–5.9 mm. The rostrum is provided with a tooth on the inferior margin, while on the superior margin one, or sometimes two, teeth are provided. The flagella of the two pairs of antennae are much increased in length. The anterior lobe of the first maxilla is in a much degenerated state; the endopodite of the second maxilliped is strikingly bent at the third segment. All the pereiopods attain much nearer the feature of the adult. The pleopods also attain to those of the adult in general appearance. The telson (Pl. VIII, Fig. 7) is in a more advanced state, and the sixth abdominal appendage is well developed and its outer branch is divided into two sections by a diaresis.

X Stage. The length of the body is 5.7–6.2 mm., measuring from the base of the rostrum to the end of the telson. The rostrum, which attains to the distal end of the second peduncular segment of the first antenna, is provided with two or three teeth on the upper margin and one on the lower margin. The width of the telson at the posterior end is narrower than that of the preceding stage. The appendages are much advanced in featnre. The first antenna bears the flagella consisting of seven and five or six articles; the flagellum of the second antenna is much elongated. In the thoracic appendages the protopodite is divided into two segments, of which the distal is fused with the proximal segment of the endopodite. The endopodite of the third maxilliped is apparently three-segmented, and in this appendage as well as in each of the anterior four pairs of legs a rudimentary epipodite is noticeable on the base.

B. Development of Appendages.

1. The First Antenna.

I Stage (Pl. VIII, Fig. 8). The peduncle is not segmented and its distal half is slightly bent outwards. The outer branch is rather short, is not articulated, and is armed with four setae near the extremity, while the inner branch is represented by a long plumous seta, which is somewhat thick at the base and gradually tapers to the distal extremity. No trace of muscles is noticeable in this stage.

II Stage (Pl. VIII, Fig. 9). The peduncle is now divided into three segments, in which the proximal is much longer than any of the others and is armed with a spine on the inner margin, while on the outer margin a seta is provided at the point where the margin is a little notched. At the articulation of each of these segments, there are some numbers of simple setae near the outer margin and a plumous one on the inner. Besides these setae the second segment is provided with one more plumous setae on the inner margin, a little behind the articulation; and on the third or the terminal peduncular segment the "antennular lobe" of GURNEY (11) is noticeable in the dorsal aspect. The outer branch or the exopodite is similar to that of the preceding stage in structure, but is a little elongated and five setae are provided near the extremity; the inner branch, however, is now considerably thickened at the base and is suddenly narrowed to the terminal plumous seta. This thickened portion is about as long as one half of the terminal seta. The muscles develope pretty well in the peduncular segments.

III Stage (Pl. VIII, Fig. 10). The peduncular segments are provided with more numbers of setae on both margins; in these setae those near the outer margin are simple and somewhat shorter in length, while those of the inner margin are plumous and are generally longer than the former. The outer branch terminates in two long setae and it is not only elongated but is somewhat constricted near the basal two-thirds of its length. In the inner branch the basal thickened portion is a little more elongated and somewhat shorter than the length of the terminal seta.

IV Stage. In the specimen obtained from the aquarium without food supply, it (Text-fig. 7) is similar to that of the preceding stage, while in the specimens which are believed to have been reared in sufficient foods or from the native pond, it is in a more advanced state (Pl. VIII, Fig. 11). The basal peduncular segment is provided with a rudimentary stylocerite, which has been noticed as a notch already in the second stage, and with two plumous setae on the inner margin; the next or the second peduncular segment has also two and the terminal has three plumous setae on the inner margin and three or four on the distal margin. The outer branch is two-articulated and terminates in four setae, of which one is stouter and longer than the others. The inner branch terminates in two setae, a long and a short. No muscle is noticeable in either of the branches.

Text-fig. 7. 1st antenna of the abnormal specimen.

V Stage. The setae on the peduncle are increased in number. In

the outer branch which consists of two articles, the terminal is distally increased in length and the two shorter setae are a little behind the extremity and both are on the inner margin. The inner branch is still the same as that of the preceding stage, but is now terminally provided with three setae, a long one and two shorter ones.

VI Stage (Pl. VIII, Fig. 12). The setae on the peduncular segments are more numerous and the stylocerite is in a more advanced state. The outer branch is now three-articulated and its distal extremity is somewhat obliquely pointed; one seta is on the extremity and one more is a little further back in position. The inner branch is now divided into two articles and bears two setae on the distal end.

VII–X Stages. In the seventh stage (Pl. VIII, Fig. 13) the terminal article of the outer branch is elongated and two tubular hairs are provided at a point a little behind the extremity, where it is articulated in the next stage, thus the two branches consist of a gradually increased number of articles, that is, the outer branch is three-, four-, five-, and seven-articulated, while the inner is two-, three-, four-, and five- or six-articulated in the VII, VIII, IX, and X (Pl. VIII, Fig. 14) stages respectively. On the other hand the peduncular segments are armed with more numbers of setae and the stylocerite develops gradually in the later stages.

2. The Second Antenna.

I Stage (Pl. VIII, Fig. 15). It consists of the basal segment or the protopodite and two branches, the outer and the inner. The basal segment is rather long and terminally provided with a minute spine on the inferior surface. The outer branch, which is foliaceous in shape, is provided with thirteen plumous setae on the margin; of these setae two are on the outer margin, while the remaining ones are on the anterior and inner margin; besides these there is a small simple seta near the distal end of the outer margin. The margin between this seta and the anterior one on the outer margin is a little concaved while arising from this margin there are three transverse lines indicating the plication of the scale. The inner branch which is as long as one half of the outer branch is thick at the base and gradually tapers to the extremity, near which three small setae are arranged in longitudinal series. Of these three setae the posterior one is very long and plumous while the anterior two are short and simple. Two bundles of the muscles are noticeable in the basal segment attaching to the base of the outer branch, in which a muscle is developed.

II Stage (Pl. VIII, Fig. 16). The basal segment is now decreased in length, while the two branches are somewhat elongated. In the outer branch the three transverse lines, which have indicated the plication of the scale in the preceding stage, now disappear. On the outer margin there are three setae; and these are not only somewhat retracted in position but are more or less decreased in length, and now they are all simple. On the anterior and inner margin there are fourteen plumous setae. The inner branch is similar in shape to that of the preceding stage, but the terminal three setae decreasing in length are now spinular in shape. The muscles develop well. In the basal segment two small muscles attaching to the base of the inner branch develop, besides the two which attach to the base of the outer branch. In the foliaceous outer branch one more small muscle appears besides the larger one which has been developed in the preceding stage.

III Stage (Pl. VIII, Fig. 17). The basal segment is short; in the two branches the outer is elongated, while the inner is somewhat contracted in length, therefore the former is a little longer than twice the length of the latter. Moreover the former is provided with an acutely pointed spine at the distal extremity of the outer margin instead of the setae which have been provided on the outer margin in the preceding stage, and its antero-inner margin exceeds the tip of the said outer spine and is provided with sixteen plumous setae on its margin,—thus it is now an ordinary scaphocerite in form. The inner branch is now terminally bluntly rounded and is devoid of the terminal setae.

IV Stage. In the specimen obtained from the aquarium, (Text-fig. 8) the inner branch is not articulated and its distal end is provided with two or three setae. In the ordinal specimen (Pl. VIII, Fig. 18), however, it distinctly differs from the former in feature. The tooth on the basal segment is increased in size. The outer branch or the scaphocerite is provided with eighteen plumous setae on the margin. The inner branch which is longer than one half the length of the scaphocerite is divided into the basal peduncular segment and the terminal flagellum. The latter is about as long as three and a half times of the former, is not articulated, and bears a small seta on its distal end.

Text-fig. 8. 2nd antenna of the abnormal specimen.

V Stage. The scaphocerite is as in the preceding stage, but is provided with about twenty two marginal setae. In the inner branch, which is now about as long as six-sevenths of the outer, the peduncular

segment bears a four- or five-articular flagellum, and on its distal extremity there are three or four small setae.

VI Stage (Pl. VIII, Fig. 19). The scaphocerite is nearly similar in feature with that of the preceding stage, while the inner branch is elongated and exceeds the tip of the scaphocerite by about the length of one terminal segment. The peduncle is provided with a seta on the distal end and bears the flagellum, which consists of eight articles and is provided with two or three small setae at the distal extremity and with one or two near the articulation of each of some intermediate segments.

VII–X Stages. In later stages the flagellum increases in length and in the number of the articles. In the seventh stage (Pl. VIII Fig. 20) it exceeds the extremity of the scaphocerite by about the length of the terminal two articles, while the number of the articles is not increased in this stage. In the next or the eighth stage, however, the flagellum consists of twelve articles and exceeds the extremity of the scaphocerite by about the length of the terminal five articles. In the peduncular segment a pair of muscles appears in this stage. In the ninth stage (Pl. VIII, Fig. 21) the flagellum is almost as long as three times the length of the scaphocerite and it consists of about seventeen articles. In the next stage it is more increased in length and in the number of articles.

3. The Mandible.

The mandible is simple in structnre and devoid of the palp. In the course of its development we are unable to recognise any characteristic changes as in that of the next species. It consists of a more or less bending stalk and a cutting portion; the inner margin of the latter is the cutting edge.

I Stage (Pl. VIII, Fig. 22). It is very weak and the cutting edge is divided into two lobes, the anterior and the posterior. The anterior is provided with four teeth, one of which is a little separated from the others, while the posterior is more or less tuberculated, but the tubercles are not so distinctly produced as to call them teeth or spines. Between these two lobes there is a deep notch, on the margin of this notch a terminally rounded tubercle arises near the posterior lobe.

II Stage (Pl. VIII, Fig. 23). The cutting edge is somewhat more complex in structure than that of the preceding stage. In the teeth of the anterior lobe the solitary one in the preceding stage transforms to a terminally branching spine, and the posterior lobe well developed is provided

with two or three branching spinular processes on the posterior margin. The terminally rounded tubercle in the first stage seen on the margin of the notch now transforms to two acute spines. Besides these armatures on the margin, some sculptures are noticeable on the inner surface near the margin, which has been almost smooth in the preceding stage.

III Stage (Pl. VIII, Fig. 24). The terminally branching spines on the cutting edge are now five or six in number; among these the anterior one is separated from the others and probably corresponds to what has been seen in the preceding stage; the remaining spines are, therefore, appearing in this stage for the first time, but two have been seen as simple spines in the preceding stage. Besides these there are some more setae on the cutting edge. The branching spinular processes on the posterior margin seen in the preceding stage disappear entirely, probably owing to their transformation into the tubercles or sculptures on the inner surface. Similar facts are noticeable in the later stages in the course of the development and aroused my interest. As the development advances the mandible is provided with more numbers of spines or teeth on the cutting edge, and these spines or teeth transform to the sculptures of the inner surface; in a similar way the mandible attains to the feature of the adult.

4. The First Maxilla.

I Stage (Pl. VIII, Fig. 25). It consists of three lobes, the anterior, the middle, and the posterior; and these lobes are close together. The anterior lobe is short and terminates in a strong spine, which is terminally curved forwards and on its outer margin there are three or four branching setae. At the base of this strong spine two somewhat slender setae are inserted on the anterior margin. The other two lobes are provided with some stout spines on the inner margin, and the spines of the middle lobe are six, while those of the posterior are three or four in number. No muscle is noticeable in this appendage.

II–VII Stages. In the second stage (Pl. VIII, Fig. 26), the three lobes are not so close together as those of the preceding stage, and the anterior lobe elongates at the point where the two slender setae are inserted, so that these two setae are on the extremity, while the strong one is on the inner margin of this lobe. One more seta arises at the base of the said strong seta; the latter is strongly curved forwards and is provided with more numbers of branching setae on its outer margin. The remaining two lobes are similar to those of the preceding stage, but the spines on the

inner margin increase in number, i. e., more than ten on the middle and five or six on the posterior. A muscle is noticeable in the anterior lobe, and it seems to be attached to the base of the strong spine. In the later stages as far as to the eighth stage it is similar in feature, but in some cases, for instance in the fifth stage, the slender seta at the base of the strong one of the anterior lobe disappears. In ordinary cases, however, it retains a similar feature still in the seventh stage.

VIII Stage (Pl. VIII, Fig. 27). The anterior lobe terminating in two setae bears a strong seta, and a slender one which has been noticeable in the preceding stages now disappears. The said strong seta is now not so strongly curved forwards as that in the preceding stage and is provided with some numbers of branching setae on both margins. The middle and the posterior lobes are furnished with more numbers of spines on the inner margin. Besides the muscle attaching to the base of the strong seta of the anterior lobe, there is now one more in the posterior lobe.

IX Stage (Pl. VIII, Fig. 28). The extremity of the anterior lobe is now provided with a single small seta and is not projected so far forward as that of the preceding stage, therefore, the lobe seems to be terminally truncated; at the inner corner of this truncation, the seta which is much smaller than that of the preceding stage is provided and on both its margins there are a less number of branching cilia. The muscle in the posterior lobe is somewhat degenerated.

X Stage (Pl. VIII, Fig. 29). The two setae on the anterior lobe are now small in size and both are now simple. The muscles in this stage are two in number, one in the anterior lobe and the other is attached to the base of the same lobe.

5. The Second Maxilla.

I Stage (Pl. IX, Fig. 30). It consists of five lobes, of which the outer is foliaceous in shape and provided with eight marginal setae. The next or the anterior lobe is long, reaches almost to the extremity of the outer lobe, and is armed with five marginal setae, of which two are on the distal end and the remaining three are on the inner margin. The succeeding two lobes are rather small in size and are provided with three and two setae on the inner margin respectively. The basal lobe is distinctly larger than any of the foregoing two, and is armed with about eight setae on the rounded inner margin. In lifetime the larva moves this appendage very vigorously; and at its base two muscles are provided, while no muscle is noticeable in this appendage.

II Stage (Pl. IX, Fig. 31). The outer lobe developing well, is prolonged posteriorly and is armed with a rather strong posteriorly directing seta; on the margin of this lobe there are nineteen or twenty setae. In these setae the anterior ones are longer than the posterior, though the said backwards directing one is long and stout. The anterior lobe, which is now distinctly shorter than the outer lobe, is provided with four setae on the inner margin, in these setae the proximal two arise from the same point. The basal lobe in the preceding stage is now divided into two lobes, therefore, there are now four lobes behind the anterior. A muscle arising near the centre of this appendage directs forwards and outwards and it enters into the foliaceous outer lobe, in which it is indistinctly divided into two bundles.

III Stage (Pl. IX, Fig. 32). The outer lobe is now provided with two or three more numbers of marginal setae. The anterior lobe is as that in the first stage and is provided with three setae on the inner margin, but it seems to be indistinctly articulated at the point where the setae arise. In the posterior four lobes the second one which has appeared in the preceding stage now becomes somewhat smaller.

IV Stage (Pl. IX, Fig. 33). The outer lobe is provided with more than thirty marginal setae, of which two or three are on the inner margin of the posteriorly producing lobe. The anterior lobe is as that of the preceding stage, but the articulations, which have been noticeable in the preceding stage, now disappear entirely. In the other lobes we are unable to recognise any points differing from those of the preceding stage.

V–VII Stages. In the fifth stage (Pl. IX, Fig. 34), the outer lobe is now provided with two strong setae at the posterior end. The anterior lobe is considerably expanded inwards at the proximal part. The proximal seta of this lobe is now on this expansion, on which one more is noticeable in this stage. The muscle which appeared in the second stage is now divided into two bundles, besides these another muscle arising near the centre of the outer lobe runs forwards and inwards into the anterior lobe, crossing the former two muscular bundles. In a like state it passes through to the eighth stage.

VIII Stage (Pl. IX, Fig. 35). The outer lobe is now provided with about thirty five marginal setae, of which four are on the inner margin of the posterior process. The expansion at the base of the anterior lobe is transformed into a peculiar lobe just before the succeeding lobe and bears three marginal setae.

IX Stage (Pl. IX, Fig. 36). The outer lobe is provided with about

forty marginal setae, of which five are on the inner margin of the posterior process. The setae on the anterior lobe are generally decreased in length, but the anterior one on the inner margin is still long, and those on the peculiar lobe are now reduced to one in number. The remaining lobes are now three in number, while in the preceding stage, as has been described above, they were four in number; owing to the fact that the posterior two are probably united to one lobe, though, I think, there may be some doubt in this case.

X Stage (Pl. IX, Fig. 37). The outer lobe developing well is provided with about fifty marginal setae, of which six or seven are on the inner margin of the posterior process. In the anterior lobe considerable deformation in feature occurs in this stage. Two setae are on the inner margin, but the others disappear entirely. The succeeding three lobes are well developed and each is provided with numerous setae near the inner margin, while the peculiar lobe between the anterior and the posterior lobes is now somewhat concaved at the margin and is devoid of the marginal setae. Thus in the later stages the outer lobe develops and is armed with more numbers of the marginal setae, while the setae on the anterior lobe degenerate.

6. The First Maxilliped.

All the appendages behind this pair are similar in feature and typical of Crustacea; each is distinctly divided into three portions, the protopodite, the exopodite and the endopodite.

I Stage (Pl. IX, Fig. 38). The protopodite is rather large in size, and its undulated inner margin is indistinctly divided into two parts armed with numerous numbers of setae. The exopodite, which is about twice the length of the endopodite, terminates in two long setae; besides these there are two setae, a long one and a short one, on each of the margins. The longer is almost in contact with the terminal one, while the shorter is at a short distance behind the extremity. The endopodite consists of four segments and terminates in four setae, two long and two short. In these four segments of the endopodite the proximal two are subequal in length and the next is the longest of all, while the terminal is the shortest. The longest or penultimate segment is armed with a seta near the middle of its inner margin and the foregoing second segment is also provided with a seta near the distal end of the inner margin, while the basal segment is dilated inwards and is provided with three setae on its inner dilation. Two muscles

are visible in the protopodite and attach to the base of the exopodite; in the latter a longitudinal muscle is noticeable.

II Stage (Pl. IX, Fig. 39). The inner margin of the protopodite is not so distinctly undulated as that of the preceding stage. In the segments of the endopodite, the penultimate is somewhat shorter and is provided with two setae near the distal end of the inner margin, while the terminal segment is increased in size and bears three setae, two stout ones and a slender one. Attaching to the base of each segment of the endopodite two rudimentary muscles are noticeable.

III Stage (Pl. IX, Fig. 40). The protopodite is now quite distinctly divided into two portions though the proximal is very small in size and its inner margin is rounded. The endopodite is now provided with two setae on the outer margin: one is near the distal end of the basal segment and the other is somewhat smaller in size and is near the base of the distal segment.

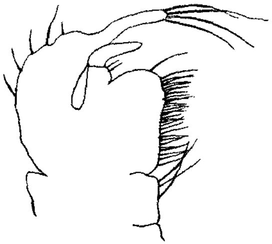
IV Stage (Pl. IX, Fig. 41). The endopodite is apparently five-segmented, though the articulation with the protopodite is somewhat obscure now. In this endopodite, as no muscle is attached to the base of the terminal segment and there is no seta on the penultimate segment, the terminal segment of the preceding stage is probably now divided into two segments. The setae on the outer margin in the preceding stage are now degenerated.

V Stage (Pl. IX, Fig. 42). Of the five segments of the endopodite seen in the preceding stage, the terminal two segments are united into one segment and on the other hand the proximal segment is fused with the terminal lobe of the protopodite, while the latter is now dilated inwards. Therefore, the endopodite now seems to consist of three segments, the terminal of which produces a narrow process which bears three setae on the extremity and one more a short distance behind this end, the penultimate is provided with a seta and the proximal with three setae on the inner margin. The muscles of the endopodite are degenerated and a rudimentary one is noticeable on the base of each of the terminal two segments.

VI–VII Stages. In the sixth stage (Pl. IX, Fig. 43), the endopodite is similar to that of the preceding stage, but all the muscles of the endopodite are degenerated now. In the exopodite a small seta on any margin is much degenerated in length or sometimes it is not noticeable at all, while near the base of the outer margin another small seta arises. In the similar feature it passes through to the next stage.

VIII Stage (Pl. IX, Fig. 44). In the protopodite the anterior lobe is now divided into two parts, of which the terminal bears four or five setae on the inner margin and corresponds to what has fused with the terminal lobe of the protopodite in the fifth stage and, I suppose, has its origin in the endopodite; while the remaining proximal portion is more dilated inwards. The longitudinal muscle in the exopodite is somewhat degenerated, and one, or sometimes two setae are provided near the base of the outer margin.

IX Stage (Pl. IX, Fig. 45). The proximal segment of the endopodite in the preceding stage is fused with the distal part of the protopodite and makes a peculiar lobe; on its inner margin four or five setae are provided; while the remaining part of the anterior lobe of the protopodite is more dilated inwards and forwards. In the endopodite, the terminal setae are now two in number and are decreased in length. The exopodite, which is terminally rounded and bears four setae, is dilated outwards at the base and on this outer dilation there are five or six marginal setae. At the base of this dilation there is a rudimentary epipodite. In a specimen I examined, which is ascertained to be in this stage, this appendage is much different in structure from the ordinary ones as shown in the Text-fig. 9; i. e., the unsegmented endopodite is devoid of setae and is articulated from the proximal part; the latter corresponds to the peculiar lobe in the ordinary specimens.


Text-fig. 9. An abnormal feature of 1st maxilliped, IX stage.

X Stage (Pl. IX, Fig. 46). In the endopodite the articulation is not noticeable in this stage, and the terminal setae decreasing in length are spinular in shape, while those on the inner margin are still long. The anterior lobe of the protopodite is much dilated, and the basal part of the exopodite is also more dilated outwards, on the margin of this outer dilation there are ten or more setae. The epipodite is well developed. The muscles are more or less degenerated in the terminal parts, but those of the proximal part are rather well developed.

7. The Second Maxilliped.

I Stage (Pl. IX, Fig. 47). The protopodite is somewhat undulated at the inner margin, and provided with less numbers of setae on the margin as compared to that in the corresponding stage of the preceding pair of appendages. The exopodite is similar in feature to that of the preceding pair, and exceeds the tip of the endopodite by about one-third of its length. The endopodite is somewhat stout and consists of four segments, in which the proximal and the terminal are subequal in length and are the shortest, while the penultimate is longer than any of others. On the inner margin of these segments some numbers of setae are provided, that is, the proximal has three, the second has one, and the penultimate has two setae; while the terminal segment is provided with four setae on the distal end and with one more small seta near the base of the outer margin. Attached to the base of exopodite two muscles are noticeable in the protopodite; in the former two longitudinal ones are noticeable. On the base of each segment of the endopodite two rudimentary muscles are attached.

II Stage (Pl. IX, Fig. 48). In the endopodite, which is somewhat decreased in length and as long as three-fifths of the exopodite, the penultimate segment in the preceding stage is now divided into two segments, therefore, the endopodite consists of five segments. In these segments the antepenultimate is devoid of setae, while the others are provided with setae just as those of the preceding stage. The setae on the inner margin of the protopodite are somewhat increased iu number.

III–VII Stages. In the third stage the setae on the inner margin of the protopodite, decreasing in number, are oriented to four groups; and in the endopodite the third segment bears a single seta on the inner margin, the others are as in the preceding stage; while on the outer margin one seta is provided on the basal segment. In a similar feature it passes through the succeeding four stages.

VIII Stage (Pl. IX, Fig. 49). The protopodite bears a decreasing number of setae on the inner margin and its outer margin is somewhat dilated at the base of the exopodite; this outer dilation is a rudiment of the epipodite. In the five segments of the endopodite, the terminal bears about six setae on the extremity; and each of the remaining four segments bears a seta on the outer margin. The penultimate segment bears a seta on the distal margin besides one on the inner and on the outer margin. In the muscles of the endopodite, two in the basal and one in the second segment are degenerated.

IX Stage (Pl. IX, Fig. 50). The protopodite is divided into two lobes, anterior and posterior; and their inner margins bear two or three setae. The rudimentary epipodite is more developed. In the segments of the endopodite, the proximal is partly united with the anterior lobe of the protopodite; the third segment bending strikingly inwards articulates the next segment at its inner margin; and the terminal two segments are now very broad. The setae on the inner margin of the endopodite generally disappear now, while on the outer margin, one seta is on the proximal segment and two on the second. The penultimate segment is armed with four setae and the terminal with numerous numbers of setae on its broad extremity.

X Stage (Pl. IX, Fig. 51). The epipodite develops well. On the inner margin of the segment, which is made of two segments, the terminal of the protopodite and the proximal of the endopodite, there are two or three setae; and the exopodite articulates at the outer margin of this segment. The setae on the outer margin of the succeeding segments of the endopodite are increased in number.

8. The Third Maxilliped.

I Stage (Pl. IX, Fig. 52). The protopodite is provided with three setae on the inner margin. The exopodite, which is a little shorter than the endopodite, is provided with four long setae near the extremity and with a shorter one a short distance behind the extremity on each margin, as we have seen in those of the preceding pairs of appendages. The endopodite consists of four segments, in which the penultimate is longer than any of the others, and the remaining three segments are subequal in length. On the inner margin there are two, one, and two setae on the first, the second, and the third segment respectively, near the extremities. The fourth or the terminal segment is provided with three setae, one stout and two slender, at the extremity, besides these a small one is near the base of the outer margin. Two strong muscles are in the protopodite attaching to the base of the exopodite, in which there are two longitudinal muscles. Attaching to each segment of the endopodite there are two small muscles.

II–IV Stages. In the second stage (Pl. IX, Fig. 53), the protopodite bears four or five setae on the inner margin. In the endopodite the penultimate segment is divided into two segments, therefore the endopodite is now five-segmented, and the setae on each segment are as in the preceding stage, so that the third segment is devoid of setae. On the outer margin the

second segment is provided with a seta near the distal extremity. In a similar feature it passes through the succeeding two stages.

V–VII Stages. In the fifth stage (Pl. IX, Fig. 54), the protopodite is provided with two or three setae on the inner margin. The endopodite exceeds the tip of the exopodite by the lengths of the terminal segment and a part of the penultimate. Each of the first and the fourth segments is provided with two setae near the distal end of the inner margin, while the second has a seta on the inner margin. On the outer margin, each of the second and the third bears a seta. In a similar feature it passes through to the eighth stage.

VIII Stage (Pl. IX, Fig. 55). The protopodite, which is indistinctly divided into two segments, is provided with a rudimentary tubercle on the outer margin and four or five setae on the inner margin. The endopodite exceeds the tip of the exopodite by the length of the terminal two segments, and its basal segment bears a seta on the inner margin, sometimes one more small seta near the middle of this margin. In the second segment the seta on the inner margin is degenerated, while one on the outer margin is still noticeable. The succeeding two segments bear now more numbers of setae near their distal extremities on both margins, and in the terminal segment the terminal strong seta is somewhat decreased in length. The muscles are distributed in the same way as those of the preceding stages, but those in the basal segment of the endopodite are almost degenerated.

IX Stage (Pl. IX, Fig. 56). The protopodite is provided with five setae on the inner margin, and the tubercular process on the outer margin is more developed. The exopodite scarcely attains to the middle of the antepenultimate segment of the endopodite and terminates in four long setae as well as that of the preceding stage, but the small seta on each margin is almost degenerated. In the segments of the endopodite the proximal two are fused to a single segment and provided with three setae, of which one is at the distal end of the outer margin and the remaining two are on the proximal part of the inner margin. The next segment bears four or five setae near the distal extremity of both margins. The next or the penultimate segment bears about six setae on the inner margin and two on the outer. In the terminal segment, which is now rather robust in shape, the terminal setae are much decreased in length. The muscles are generally more or less degenerated in this stage.

X Stage (Pl. IX, Fig. 57). The protopodite bears more numbers of setae on the inner margin, and the epipodite develops at its outer margin. The exopodite exceeds the extremity of the proximal segment of the endo-

podite for a short distance, and bears four long setae at the distal end. In the endopodite the articulation between the terminal two segments disappears now, so that the endopodite consists of three segments; the proximal is provided with four or five setae in the inner margin and most of them are on the proximal part, the next with four setae of which three are on the inner margin and another is at the distal end of the outer margin. The terminal segment, which bears a strong seta and four slender ones on the distal end, is provided with many setae on the inner margin and with two or three on the outer margin.

9. The First Pereiopod.

I Stage (Pl. IX, Fig. 58). It is not segmented and is represented by a terminally two-branched sac. In these two branches, the inner is terminally provided with a single seta and is longer than the outer, which is provided with two setae at the distal end.

II–IV Stages. In the second stage (Pl. IX, Fig. 59), it is much increased in length compared to that of the preceding stage and is distinctly segmented. The protopodite bears four setae on the inner margin; the exopodite, which scarcely reaches to the distal end of the penultimate segment of the endopodite, is similar in structure to the preceding pair of appendages; i. e., it terminates in two long setae and on each margin there are two setae, a longer and a shorter; the longer is nearly as long as the terminal and is almost in contact with it, while the shorter is a short distance retracted from the extremity in position. The endopodite consists of five segments, in which the second and the penultimate are subequal in length and each is somewhat longer than any of the others. On its inner margin two setae are provided on each of the proximal and the penultimate segment, and one on the second; while on the outer margin one is on each of the proximal two segments. On the terminal segment, three setae, one strong and two slender, are provided at the distal end, and another small seta is near the base of the outer margin. Two strong muscles attach to the base of the exopodite; in the latter two longitudinal ones are noticeable. On each of the segments of the endopodite two small muscles are attached at the base. In a similar structure it passes through the succeeding two stages.

V Stage (Pl. IX, Fig. 60). In this stage the endopodite exceeds the tip of the exopodite by the lengths of the terminal and a part of the penultimate segment. On the inner margin of the endopodite, a setae appears

on the third segment in this stage; while on the outer margin the setae of the second are two in number and the penultimate, which is now a little dilated at its inner distal corner, is provided with one. The terminal segment is somewhat tuberculated at the base of the terminal setae.

VI–VII Stages. In the sixth stage (Pl. IX, Fig. 61), some setae of the endopodite are degenerated; i. e., one outer setae of the second segment is degenerated and the third segment is now devoid of setae on the inner margin. The penultimate segment is considerably dilated inwards and forwards so as to form the immovable finger of the chela. On the distal end of the terminal segment there is a strong seta, and one slender one on the outside and two slender setae are provided, on the inner margin. In the next stage it is similar in feature.

VIII Stage (Pl. IX, Fig. 62). The endopodite is a little more elongated than that of the preceding stage. On the inner margin of the endopodite, one seta near the distal end of the third segment and another near the middle of the penultimate segment appear; and on the latter segment two setae are at the distal end of the outer margin. The protopodite bears two or three setae on the inner margin and is somewhat dilated at the outer margin.

IX Stage (Pl. IX, Fig. 63). The protopodite is indistinctly divided into two segments, and bears three or four setae on the inner margin; the dilation on the outer margin is more distinct now. The endopodite exceeds the extremity of the exopodite by the lengths of the terminal two segments and a part of the foregoing segment; its proximal segment bears a seta on the inner margin, the second two on the outer, and the third also two on the outer margin. At the extremities of the two fingers of the chela there are increased numbers of setae.

X Stage (Pl. IX, Fig. 64). The process on the outer margin of the protopodite develops to a rudimentary epipodite, and its inner margin bears five or six setae. The exopodite, which attains to the middle of the third segment of the endopodite, is provided with one or two small setae near the base and is devoid of setae near the distal end, so that on the terminal part there are only four long setae. The setae at the end of the two fingers are much increased in number.

10. The Second Pereiopod.

I Stage. It is sac-like in feature and is terminally divided into two branches as well as the preceding pair, but the extremities of the branches have no setae in this pair.

II–IV Stages. In the second stage (Pl. IX, Fig. 65), it is now distinctly segmented. The protopodite bears four setae on the inner margin, and the exopodite which scarcely reaches to the distal end of the penultimate segment of the endopodite is just like that of the preceding pair in feature. The endopodite consists of five segments, in which the proximal and the penultimate are subequal in length and each is somewhat longer than any of the remaining three. On the inner margin, each of the proximal and the penultimate segment has two setae, the second has one, and the third is devoid of seta; while on the outer margin the penultimate segment bears a seta near the middle. The terminal segment bears two setae, a stout and a slender, at the extremity and another small one near the base of the outer margin. Two muscles are attached to the base exopodite, in which two longitudinal ones are noticeable. Attaching to the base of each segment of the endopodite two muscles are provided. In a similar feature it passes through the succeeding two stages.

V–VII Stages. In the fifth stage (Pl. IX, Fig. 66), each of the second, the third, and the fourth segment of the endopodite bears a seta near the distal end of the inner margin; while on the outer margin, the proximal and the penultimate segment are each provided with two setae, the latter segment being somewhat dilated at its distal inner corner. In the terminal segment, one more slender seta is added at its extremity, and the one near the base of the outer margin disappears. In the later stages the endopodite increases in length compared to the exopodite, and the penultimate segment of the endopodite is more dilated at the distal inner corner and forms the immovable finger of the chela.

VIII Stage (Pl. IX, Fig. 67). The endopodite exceeds the tip of the exopodite by the length of the terminal two segments. Each of the proximal three segments bears a seta on each margin near the distal end. The penultimate segment is distally more elongated at the inner corner, and is provided with two slender setae at the distal end of this prolongation, with two more near the middle of the inner margin, and another two at the distal extremity of the outer margin. The terminal segment bears four setae, a stout and three slender, at its extremity. The muscles in the proximal segment of the endopodite are more or less degenerated.

IX Stage (Pl. IX, Fig. 68). The protopodite is indistinctly divided into two segments and dilated to a process on the outer margin. This process is a rudiment of the epipodite. The exopodite terminating in four long setae is devoid of the marginal small setae and extends a little beyond the extremity of the second segment of the endopodite. In the endopodite,

the proximal segment is provided with a seta on the inner margin, the second and the third each have one on the outer margin, while the other setae now disappear. The fingers of the chela are well developed and are provided with many setae on their extremities.

X Stage (Pl. IX, Fig. 69). The epipodite is now fully developed. The exopodite scarcely reaches to the distal end of the second segment of the endopodite; in the latter the carpal segment is excavated at the distal extremity of the outer margin. The fingers of the chela are well developed, and many setae are provided on their terminal portions.

11. The Third Pereiopod.

I Stage. It is similar in feature to the preceding pair of appendages in the corresponding stage, but somewhat smaller in size.

II–V Stages. In the second stage (Pl. IX, Fig. 70), it is now distinctly segmented. The protopodite is provided with three setae on the inner margin. The exopodite, which attains to the middle of the penultimate segment of the endopodite, terminates in a similar manner to those of the preceding pairs of the thoracic appendages. The endopodite consists of five segments, each of which is subequal in length, but is slightly increased in length to the terminal. On the inner margin the proximal and the fourth segment are each provided with two setae, and the second with one; while on the outer margin a single seta is provided on the third segment. The terminal segment is provided with two setae on the extremity, a slender and a stout; and has another small one near the base of the outer margin. Two strong muscles are attached to the base of the exopodite, in which there are two longitudinal ones. Attaching to the base of each segment of the endopodite are two small muscles. In a similar feature it passes through the succeeding three stages, but in the third stage an additional seta appears on the outer margin of the second segment of the endopodite.

VI Stage (Pl. IX, Fig. 71). The endopodite is elongated and exceeds the extremity of the exopodite by the length of the two terminal segments. In the segments of the endopodite the second and the fourth are much elongated and are subequal in length, each being much longer than any of the others; in the latter the terminal is somewhat longer than either of the remaining two. Each of the first, the third, and the fourth segments is provided with two setae on the inner margin, while the second has a single seta. On the outer margin, however, a seta is provided on each of the

second and the third segments, while the fourth bears two setae at the distal end. The muscles of the proximal segments seen in the preceding stages are somewhat degenerated.

VII Stage (Pl. IX, Fig. 72). The protopodite, which is indistinctly divided into two segments, bears six or seven setae on the inner margin and is more or less dilated at the outer margin. On the inner margin of the endopodite, the second segment is devoid of setae, the third has one, and the fourth has one near the middle of the margin and two more setae at its distal end. The terminal segment bears a stout and three or four slender setae at the distal end, while the small one on the outer margin has disappeared.

VIII Stage (Pl. IX, Fig. 73). In the endopodite, which exceeds the extremity of the exopodite by the length of the two terminal segments and a part of the preceding, each of the third and the fourth segment is provided with a small seta at the distal end of the inner margin and the latter segment has one more near the middle of this margin; the setae on the outer margin are distributed similarly to those of the preceding stage. The muscles of the proximal segments of the endopodite are somewhat degenerated.

IX Stage (Pl. IX, Fig. 74). The outer dilation at the outer margin of the protopodite is more developed. The endopodite is elongated, while the exopodite is somewhat decreased in length, therefore, the former exceeds the tip of the latter by almost the length of the four terminal segments of the former. The setae on the inner margin of the endopodite are mostly degenerated, but one is near the distal end of each of the third and the fourth segments, and in the latter segment two spinules are provided on its inner margin; while the setae on the outer margin are similar to those of the preceding stage. The terminal segment terminates in a short stout seta, at the base of which two or three short setae are provided.

X Stage. The epipodite develops on the outer margin of the protopodite. Each of the proximal two segments of the endopodite bears a spinule near the middle of its inner margin, and the third segment now bears one more seta near the middle of the outer margin. On the penultimate segment two more setae are noticeable on the outer margin besides those seen in the preceding stage. In the terminal segment three or four spinules are provided in the place where some numbers of setae have been bestudded in the preceding stage.

12. The Fourth Pereiopod.

I Stage. It is represented by a small tubercle, which is not terminally branched into two lobes.

II Stage. It is now well developed and terminally divided into two branches, but is not segmented.

III Stage (Pl. X, Fig. 75). It is now distinctly segmented. The protopodite bears a seta on the inner margin. The exopodite, which scarcely exceeds the distal end of the third segment of the endopodite, is terminally provided with two or three setae presents an appearance different from that of any preceding pair of appendages. The endopodite consists of five segments, in which the penultimate is longer than any of others, which are subequal in length. On the inner margin, the proximal is provided with a seta and the penultimate with two, while on the outer margin no setae are noticeable. The terminal segment of the endopodite bears two setae on the extremity, a stout and a slender. The muscles are developed as those of the preceding pairs, but they are somewhat rudimentary.

IV Stage (Pl. X, Fig. 76). The protopodite bears two setae on the inner margin. The exopodite is now ordinary in appearance; that is, it terminates in two long setae and on each margin there are two setae, a long and a short. The former is nearly as long as the terminal and is in contact with it at the base, while the latter is a little behind the terminal. In the segments of the endopodite, the proximal two are elongated and now the second is longer than the penultimate and the first is almost as long as the latter. On the inner margin, each of the first, the third, and the fourth is provided with a seta, while on the outer margin the third and the fourth have each one. The muscles are now well developed.

V Stage (Pl. X, Fig. 77). The protopodite is now provided with four setae on the inner margin. In the segments of the endopodite, the second and the fourth are subequal in length and each of the first and the fourth is provided with two setae near the extremity of the inner margin, and those on the outer margin are like those of the preceding stage. The terminal segment bears three setae, a stout and two slender, at the distal end.

VI-VII Stages. In the sixth stage (Pl. X, Fig. 78), the setae on the inner margin of the protopodite are three in number. The endopodite exceeds the extremity of the exopodite by the lengths of the terminal two segments and a part of the foregoing one, and its second segment bears a setae near the distal end of each margin. The terminal segment now bears

two setae, a stout and a slender, at the extremity, near where a small tubercle is noticeable on the inner margin. The muscles in the proximal segment of the endopodite are somewhat degenerated. In a similar feature it passes through the seventh stage.

VIII Stage (Pl. X, Fig. 79). The endopodite exceeds the extremity of the exopodite by the length of the terminal three segments; and in these segments, the third is provided with a small seta on the inner margin, and the penultimate has two or three on the inner and four or five setae on the outer margin.

IX Stage (Pl. X, Fig. 80). The protopodite is a little dilated on the outer margin. The exopodite reaches to the middle of the second segment of the endopodite and is devoid of the small setae near the extremity. In the segments of the endopodite, the second is devoid of seta on the inner margin, while the third is provided with two setae near the extremity of the inner margin and the fourth has two spinules on the inner margin in the place where setae have been bestudded in the preceding stage. In the terminal segment the terminal strong seta is decreased in length and on the inner margin of this segment many spinular setae are provided. In the muscles of the endopodite some proximal ones are degenerated.

X Stage (Pl. X, Fig. 81). The dilation on the outer margin of the protopodite is now transformed to a rudimentary epipodite, and the protopodite is divided into two segments. The exopodite does not reach to the middle of the second segment of the endopodite; in the segments of the latter, each of the proximal two segments is provided with a spinule near the middle of the inner margin besides one or two small setae, and has some setae also on the outer margin. The third segment produces at the distal outer corner a spinular tubercle, and is provided with a seta on the inner margin and with two or three on the outer. The next or penultimate segment bears some numbers of setae besides the spinules, which are sometimes three in number. On the terminal segment some numbers of spinular setae are almost uniformly distributed on the inner margin, and on the outer margin two or three setae are provided.

13. The Fifth Pereiopod.

I–II Stages. It is not well developed, and the tubercle is scarcely noticeable in the first stage. In the next stage, however, it is somewhat increased in size.

and terminally divided into two branches, in which the inner is longer than the outer, but is not segmented.

IV–VI Stages. In the fourth stage (Pl. X, Fig. 83), it is distinctly segmented. The protopodite is devoid of the marginal setae; and the exopodite scarcely reaches to the end of the second segment of the endopodite and is provided with two or three setae on the distal extremity, where it is bluntly rounded. The endopodite consists of five segments, in which the terminal two are subequal in length and each is somewhat longer than any of the others. On the inner margin the proximal and the penultimate are each provided with two setae, the second has one while the third is without setae; on the outer margin, however, each of the second and the third bears a seta. The terminal segment bears two setae, a stout and a slender, on its distal extremity. The muscles attaching to the base of the exopodite are well developed, but the longitudinal one in the exopodite is not noticeable; those attaching to the bases of the segments of the endopodite are developed, but they are still rudimentary. In a similar feature it passes through the succeeding two stages; but in the later stages some segments of the endopodite are elongated.

VII Stage (Pl. X, Fig. 84). The protopodite is provided with a seta on the inner margin. The exopodite, which still scarcely reaches to the distal end of the second segment of the endopodite, now attains to the ordinary feature, i. e., it terminates in two long setae and on each margin there are two setae, a long and a short. The longer is just behind the base of the terminal and is nearly as long as this, while the shorter is a little retracted in position. In the segments of the endopodite, the penultimate is the longest of all, the second and the terminal are subequal in length and each is longer than either the first or the third. On the inner margin the proximal segment now bears only one seta. The terminal segment is provided with a small tubercle at the base of the terminal strong seta. All the muscles are now fully developed.

VIII Stage (Pl. X, Fig. 85). The protopodite is somewhat dilated outwards at the outer margin. The exopodite scarcely reaches to the middle of the second segment of the endopodite. On the inner margin of the endopodite, two, one, and one seta are provided on the first, the second, and the third segment respectively, while the fourth bears two or three setae near the extremity. On the outer margin, this fourth segment bears four or five small setae near the distal end. In the terminal segment the terminal strong seta is decreased in length, and two spinular setae are on the inner margin, while two or three setae are on

the outer. The muscles in the proximal segment of the endopodite are somewhat degenerated.

IX Stage (Pl. X, Fig. 86). The protopodite bears three setae on the inner margin, and the outer expansion is more developed. The exopodite, which does not reach to the middle of the second segment of the endopodite, terminates in four long setae and is devoid of the marginal small ones. The terminal segment of the endopodite is provided with three spinular tubercles on the inner margin, and the terminal strong seta is now transformed to an elongated point of this segment.

X Stage (Pl. X, Fig. 87). The rudimentary epipodite is developed on the outer margin of the protopodite, which is now divided into two segments. The exopodite is a little longer than the proximal segment of the endopodite and is terminally provided with two or three setae. In the segments of the endopodite, the proximal is provided with one or two setae near the extremity of the inner margin and the second with two or three setae besides a spinule near the middle of the inner margin; on the outer margin of these segments there are some numbers of small setae. The third segment bears one on the inner and two on the outer margin, besides a spinule near the distal end. Some numbers of setae are distributed on the penultimate segment, on the inner margin of this segment three spinules are provided; on the terminal segment increased numbers of spinular tubercles are arranged on its inner margin. The muscles in the proximal segment of the endopodite and the exopodite are somewhat degenerated, while those in the protopodite are now well developed.

14. The First Pleopod.

The anterior five pairs of pleopods appear as small tubercles at first in the fourth stage, and in the next stage they are terminally two-lobed.

VI Stage (Pl. X, Fig. 88). It is not distinctly segmented, but the terminal two lobes are elongated. The inner lobe scarcely reaches to the middle of the outer, and both of them are terminally provided with two small tubercles.

VII Stage (Pl. X, Fig. 89). It is now segmented, so that, the protopodite bears terminally two branches, the exopodite and the endopodite. The exopodite increasing in length is about five times the length of the endopodite and is provided with four tubercles near the extremity, while the latter is distally obtusely rounded. The muscles are noticeable in the protopodite and in the exopodite, but they are still rudimentary.

VIII Stage (Pl. X, Fig. 90). The outer branch or exopodite is more elongated than was that of the preceding stage and is a little more than five times the length of the inner. The former is now provided with six short setae on the margin of its terminal half, while the latter is still obtusely rounded and is devoid of setae. The muscles in the protopodite are more developed than those of the preceding stage.

IX Stage (Pl. X, Fig. 91). The outer branch is nearly six times as long as the inner, and is provided with nine or ten setae on the margin; among these one or two proximal setae on the inner margin are usually smaller than the others. The inner branch is now provided with four small setae on the margin. The muscles are pretty well developed; those in the protopodite are distributed with some complexities, some are oblique while the others are longitudinal.

X Stage (Pl. X, Fig. 92). The protopodite is dilated at the distal outer corner, where two or three small setae are provided. The outer branch is a little more than six times the length of the inner and is provided with eleven marginal setae, in which the proximal two on the inner margin are smaller than the others.

15. The Second to Fifth Pleopods.

Four pairs of pleopods behind the first pair are similar in feature as well as in manner of development, though there are some differences in size; and until the fifth stage they are similar in feature to the first pair.

VI Stage (Pl. X, Fig. 93). It is not distinctly segmented as well as that of the preceding pair in the corresponding stage. The terminal inner lobe exceeds the middle of the outer; and each of these two lobes is provided with two tubercles at the distal extremity. The inner margin of the inner lobe is a little angulated near the middle.

VII Stage (Pl. X, Fig. 94). It is now distinctly segmented; the protopodite terminally bears two branches, the outer exopodite and the inner endopodite. In these branches the latter is about two-thirds the length of the former. The small tubercles are provided at the extremity of each branch, and the inner margin of the inner branch projects inwards near the middle point. A rudimentary muscle is noticeable in the protopodite.

VIII Stage (Pl. X, Fig. 95). The outer branch is provided with seven small setae on the margin while the inner has three small ones, besides these there are two rudimentary setae on the inner margin; on this margin the inner projection seen in the preceding stage develops to a

triangular tubercle, which is almost articulated from this branch. Two muscular bundles are noticeable in the protopodite and one in the outer branch.

IX Stage (Pl. X, Fig. 96). The protopodite is a little dilated at the distal outer corner, where a small seta is provided. The outer branch is provided with nine setae on the margin and the inner branch with seven marginal setae. The triangular tubercle in the preceding stage on the inner margin of the inner branch now develops into a small rod-like appendix, and on its extremity there are two processes which are terminally strongly curved. The muscles are well developed, and in the inner branch there are two muscles, one is similar to that in the outer branch and another is along the inner margin extending to the base of the appendix.

X Stage (Pl. X, Fig. 97). The protopodite is now provided with three or four small setae near the distal outer corner. The onter branch is provided with about eight to twelve marginal setae, while the inner branch has seven setae on the margin. The small appendix on the inner margin of the inner branch bears four small hooked processes. The muscles in the protopodite are somewhat complex in state, and that in the outer branch is well developed and arising from one point is expanded to the margin. There are two muscles in the endopodite similar to those of the preceding stage; one seems to correspond to that of the exopodite and is similar to that in feature, while the other belongs to the appendix or stylambris.

16. The Sixth Pleopod.

It has been described above in the place where the development of the telson is described

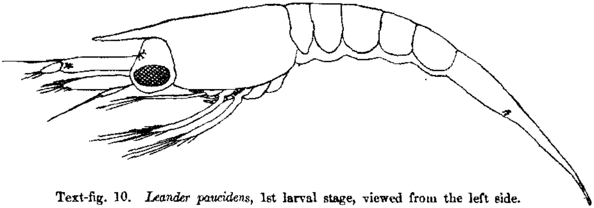
Leander Paucidens.[1]

Palaemon paucidens de Haan, 1856; *Leander paucidens*, Stimpson, 1860.

The species, belonging to the family Palaemonidae, is very common in Japan and is known under the name "Suji-yebi" or a striated prawn. It occurs in fresh and sometimes in brackish waters. It has been reported that the species is also distributed to the Kuril Isls., southern part of Korea, and Futschau in China. The prawn is distinctly larger than the preceding species; in the large specimens it reaches 7 cm. measuring from

[1] The figure of this species was given by de Haan (15).

the base of the rostrum to the end of the telson. The colour of the adult specimen is pale grayish yellow as a whole, and some dark brownish



Text-fig. 10. *Leander paucidens*, 1st larval stage, viewed from the left side.

colourations are distributed on it; i. e., on the carapace, as well as on each of the third, and the sixth abdominal segment there is a transverse band along the posterior margin. The telson and two branches of the sixth abdominal appendage are similarly coloured near the posterior end; and connecting these colourations there is a transverse band. Somewhere else on the carapace and the abdomen there are some numbers of coloured spots. The distal extremities of the segments of the thoracic appendages are yellow in colour. The spawning season of the species is similar to the species described above; some specimens bear eggs already at the end of March, and on the other hand we can find some numbers of larvae of the first stage still in the beginning of October. The egg is pretty large in size, measuring 1.75×1.28 mm., and is pale bluish coloured at first, but with the advancing development of the animal it is almost colourless except for the eye pigment.

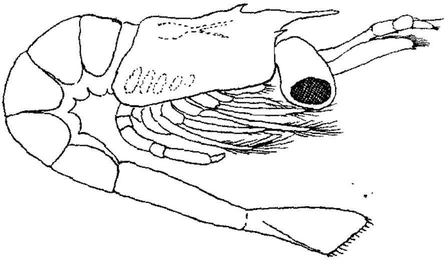
A. Metamorphosis.

I Stage (Text-fig. 10). In the stage just hatched out from the egg, the body measures 5.8 mm. in length from the base of the rostrum to the end of the telson. It is almost translucent white except the eye pigment and is flashed with very light pink colour at the tip of the peduncle of the first antenna and at the base of the fan-shaped expansion of the posterior abdominal segment but in some cases these colourations are very obscure. The larva is always vertical in position, head below and tail-fan above, and it swims towards the dorsum.

The cephalothorax is covered with the unarmed carapace, but the

rostrum projects horizontally. The abdomen now consists of six segments, as the sixth segment is coalesced with the telson; the latter is thin, much laterally dilated, and is spatular in form. (Pl. X, Fig. 98). The posterior margin of this spatular expansion is nearly straight and is armed with seven pairs of plumous setae; in these setae the outer two pairs are devoid of branching cilia on the outer margin, while in the remaining pairs of setae the cilia are provided on both margins. The muscles near the telson are not well developed; some are noticeable only as a trace. In the cephalothoracic appendages, the anterior eight pairs are pretty well developed and the functional to some extent. The peduncle of the first antenna is not segmented; and the outer foliaceous branch of the second antenna is distinctly plicated near the distal extremity. Each of the three pairs of maxillipeds having an exopodite serves the role of a swimmeret. In the pereiopods, the anterior two pairs are represented by biramous sacs, though they are not segmented; while the succeeding three pairs are imbedded in the medial line of the ventral surface. The pleopods are not developed. Two days after hatching the first ecdysis takes place and it passes into the next stage.

II Stage (Text-fig. 11). No changes in colour as well as in body length are noticeable in this stage. The carapace is armed with a tooth on the gastric region and with a pair of supraocular spines. The seventh abdominal segment or the telson is scarcely articulated with the preceding



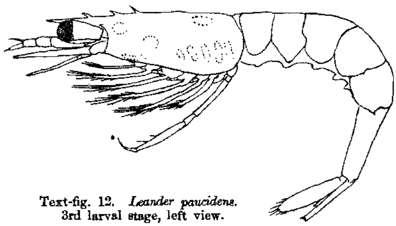
Text-fig. 11. *Leander paucidens*, 2nd larval stage, viewed from the right side.

segment and on its posterior margin there is an additional pair of small setae between the inner pair in the preceding stage; therefore, the posterior margin is now provided with eight pairs of setae (Pl. X, Fig. 99). The

muscles in the telson are a little more developed than those of the preceding stage, while the uropod or the sixth abdominal appendage is not developed.

The peduncle of the first antenna is now divided into three segments, in which the proximal is the longest and is much dilated outwards at its posterior portion. In the second antenna the outer foliaceous branch is not plicated, and the inner branch is now much elongated. The anterior two pairs of pereiopods are distinctly segmented and are similar in feature to the third maxilliped. The third and the fifth pereiopod are free from the ventral surface, but are not segmented, while the fourth pair is still imbedded in the medial line. Two or sometimes three days after the first ecdysis it transforms to the third stage.

III Stage (Text-fig. 12). The colour of the body is as in the preceding stage, but sometimes it is distinctly but slightly brownish red. The body length is 6.3 mm. measuring from the base of the rostrum to the end of the telson.



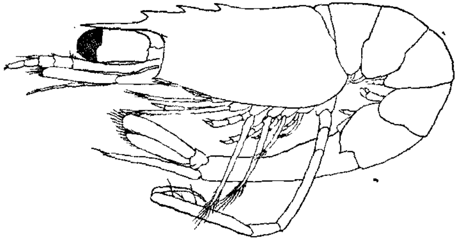
Text-fig. 12. *Leander paucidens.* 3rd larval stage, left view.

The carapace is armed with three teeth on the medial line; the newly arising teeth are in front of that seen in the preceding stage. The pleura of the abdominal segments are developed and that of the fifth abdominal segment is posteriorly pointed. The telson (Pl. X, Fig. 100) is distinctly articulated with the foregoing segment, and the posterior margin of the former is concave. The outer three pairs of setae of this margin are much decreased in length; the uropod is now developed, but is not distinctly articulated and its inner branch being rudimentary is devoid of marginal setae. The muscles are pretty well developed in the sixth abdominal segment, but those in the telson are still rudimentary and none is noticeable in the uropod.

The flagellum of the second antenna is nearly as long as the scaphocerite. The third and the fifth pair of legs are now distinctly segmented; in these two legs the former is similar in feature to the preceding pair of legs, while the latter is strikingly long and uniramous. The fourth leg is represented by a terminally two-branched sac, but is not segmented. Two

or three days after the second ecdysis the third takes place and the larva transforms to the fourth stage.

IV Stage (Text-fig. 13). The body length without the rostrum is 6.3–6.6 mm., and the colour is brownish red. I saw a larva of this stage



Text-fig. 13. *Leander paucidens*, 4th larval stage, viewed from the left side.

capturing a living larva of an insect, *Ephemera*, with its enormously long last pair of legs.

The carapace is similar in feature to that of the preceding stage, but it is now distinctly pointed at the pterygostomian angle. The telson (Pl. X, Fig. 101) diminishing in width at the posterior end is now subquadrate in shape; and the outer two pairs of the marginal setae are transmitted to the lateral margin of this segment. The sixth abdominal appendage is now well developed and is distinctly segmented; both of the branches are provided with some numbers of marginal setae; thus this appendage forms the lateral plate of the rhipidula, and two muscular bundles are in each of the protopodite, the outer branch, and the telson.

The two flagella of the first antenna are elongated, and the outer now consists of two articles while the inner is uniarticular. The flagellum of the second antenna consists of about seven articles and is a little longer than the scaphocerite. The penultimate segment of each of the first and the second pereiopods is dilated at the distal inner corner. The fourth pereiopod is now distinctly segmented and is similar in feature to the preceding pairs. The rudimentary pleopods arise as bud-like processes. Three or four days after the third ecdysis it transforms to the next stage.

V Stage (Text-fig. 14). The body length is 6.6–7.5 mm., and the colourations are somewhat orientated; generally the posterior part of the

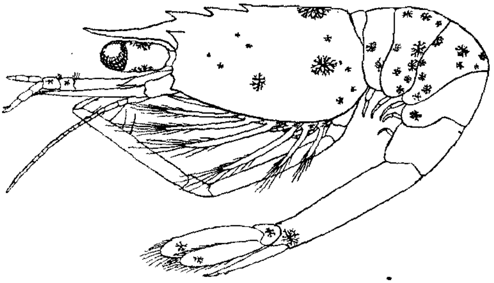
Text-fig. 14. *Leander paucidens*, 5th larval stage left view.

carapace and the anterior half of the abdomen are coloured with somewhat deep brownish red, speaking precisely, increased numbers of chromatophores are gathered on these parts.

The carapace is as in the preceding stage, but I found a specimen which has four teeth on the medial line of the carapace, as shown in the text figure. The telson (Pl. X, Fig. 102) is diminished in width at the posterior end. The sixth abdominal appendage is more developed, and its two branches are provided with increased numbers of setae. Near the base of the outer margin of the outer branch three or four small setae are provided; and the muscles in this appendage are now well developed. The flagellum of the second antenna exceeds the extremity of the scaphocerite by about half the length of this scale, and consists of eleven or twelve articles. In the anterior two pairs of legs, the distal inner dilation of the penultimate segment is elongated and forms the immovable finger of the chela. The pleopods are now distinctly segmented and the protopodite bears two branches, the exopodite and the endopodite; but these branches are provided with no marginal setae. Three to five days after the fourth ecdysis it transforms to the sixth stage.

VI Stage (Text-fig. 15). The body length is 7.4–7.9 mm., but one specimen which has been reared to this stage in the aquarium from the first stage is 6.8 mm. long. This, I know, is an extraordinary small specimen.

The carapace is similar to that of the preceding stage, but the antennal spine arises in this stage, so that the carapace is now armed with three pairs of spines, the supraorbital, the antennal, and the pterygostomian spines besides the rostrum and those in the medial line. The telson (Pl. X, Fig. 103) diminishes its width at the posterior end and its lateral



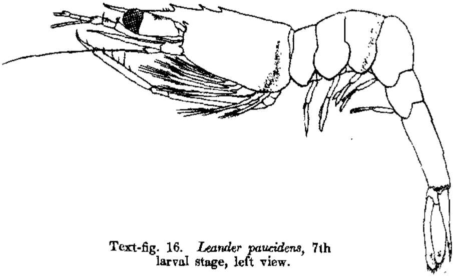
Text-fig. 15. *Leander paucidens*, 6th larval stage, viewed from the left side.

margin is distinctly convex, but the posterior margin is still concave. The marginal setae of the two branches of the sixth abdominal appendage are more or less increased in number.

In the outer flagellum of the first antenna, the terminal segment is more or less dilated at the base and is a little folded terminally; this is the rudiment of the branching of this flagellum. In the second antenna the flagellum is about one and two thirds times the length of the scaphocerite. The pleopods are more developed than those in the preceding stage, and the small appendix or the stylambris is distinctly differentiated on the inner margin of the inner branch in the second to fifth pleopod, but the marginal setae are not developed. More than three days after the fifth ecdysis, it transforms to the seventh stage.

VII Stage (Text-fig. 16). Most of the larvae reared in the aquaria died in the preceding stage, and those of this stage and the following ones were obtained by rearing the larvae of the sixth stage, which were collected from the native pond, in aquaria. The body length without the rostrum is 8.1–8.9 mm., the body colour is similar to that of the preceding stage, but the colours at the base of the third pereiopod and near the posterior end of the uropod are remarkable in this stage.

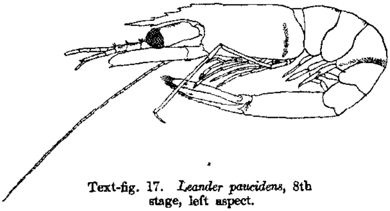
The pleuron of the fifth abdominal segment is posteriorly rounded, while in the preceding stages it has been pointed. The telson (Pl. X, Fig. 104) is much narrower at the posterior end and its posterior margin is now somewhat convex, but its marginal setae are still eight in number between the lateral strong ones. Each of the two flagella of the first antenna consists of four articles, but the outer is distinctly branched at



Text-fig. 16. *Leander paucidens*, 7th larval stage, left view.

the distal extremity of the second article. The flagellum of the second antenna is a little more than twice the length of the scaphocerite. The pleopods are well developed and some numbers of setae are provided on the margin; swinging the branches forwards and backwards the pleopods seem to act the role of swimmers. After three or four days it transforms to the next stage.

VIII Stage (Text-fig. 17). The body is distinctly decreased in size, and its length is 7.3–7.7 mm. The chromatophores on the surface of the body now make some distinct markings: a transverse band is on the posterior part of the carapace and another along the posterior margin of the third abdominal segment; the former on each side does not meet in the medial line, while the latter on each side meets in the medial line, thus both make a transverse band. These markings are light brownish red in colour. Giving up its pelagic life in this stage, it walks on the bottom with its pereiopods; so it differs in many respects from that of the preceding stage.



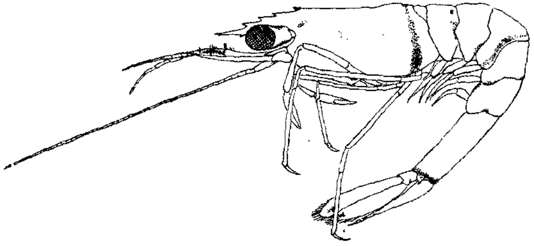
Text-fig. 17. *Leander paucidens*, 8th stage, left aspect.

The rostrum is elongated and exceeds the tip of the eye by nearly one-third of its length. The teeth on the superior margin of the carapace

seen in the preceding stage are now on the upper margin of the rostrum and are four in number; on the lower margin there is a tooth. The supraorbital teeth now disappear. The abdomen is as in the preceding stage, but the telson (Pl. X, Fig. 105) is different in some respects from that of the preceding stage; viz., on the posterior margin a pair of small setae is provided between the strong setae at the distal outer corners.

Of the two flagella of the first antenna, the inner consists of five segments and is a little shorter than the outer; while the latter is also five-segmented, and from the third segment the uniarticular branch produces. The flagellum of the second antenna is now about two and a half times as long as the scaphocerite. In the pereiopods the exopodites are much degenerated. The fifth uniramous leg, which has been inverted in position in the preceding stage, is now ordinary in position and is still much longer than the others. The marginal setae of the pleopods are increased in number. About four days after the seventh ecdysis it transforms to the ninth stage.

IX Stage (Text-fig. 18). The body length is 7.8–8.2 mm., measuring from the base of the rostrum to the end of the telson. The transverse



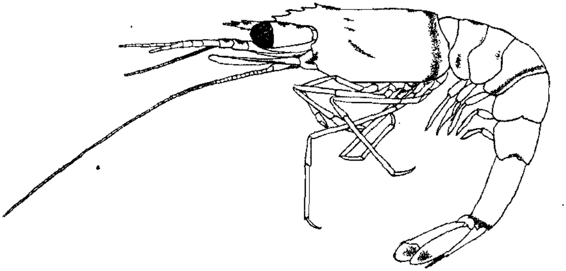
Text-fig. 18. *Leander paucidens*, 9th stage, left aspect.

bands are brown in colour, and the band on each side at the posterior part of the carapace is connected in the medial line, and both make a transverse band. Besides these bands, there are some numbers of markings on the surface; viz., on the posterior rostral tooth, one or two on the gastric region, and on the pleura of the abdominal segments, etc.

The posterior margin of the telson (Pl. X, Fig. 106) is sharply pointed in the medial line. The inner flagellum of the first antenna is a little

longer than the outer and consists of ten articles, while the outer has eight articles. The branching appendix of the latter, arising from the third segment, now consists of two articles and is terminally provided with two *aesthetes*. *The flagellum of the second antenna is* about three and a half times the length of the scaphocerite. The exopodites of the pereiopods entirely disappear, except that of the fourth which is still noticeable as a minute knob, and this leg is elongated while the fifth leg is decreased in length, therefore, the former is now not so much shorter than the latter as in the preceding stage. It transforms to the tenth stage four days after the eighth ecdysis.

X Stage (Text-fig. 19). The body is 8.4–9.0 mm. long, measuring from the base of the rostrum to the end of the telson. There are many



Text-fig. 19. *Leander paucidens*, 10th stage, left aspect.

additional pigments on the rostrum, on the carapace, and on two pairs of antennae, etc.; and these markings are somewhat darker than those in the preceding stage.

The rostrum, which reaches almost to the distal end of the peduncle of the first antenna, is armed with six teeth on the upper margin and with two on the lower. The telson (Pl. X, Fig. 107) diminishes its width at the posterior end, and the medial tooth on the posterior margin is now very conspicuous. The outer branch of the uropod is distinctly divided into two parts by a diaresis. The branching appendix on the outer branch of the first antenna arises from the third segment and consists of three articles. *The flagellum of the second antenna is almost* four times as long as the scaphocerite. No trace of the exopodite is noticeable on the pereiopods.

B. Development of Appendages.

1. The First Antenna.

I Stage (Pl. XI, Fig. 108). The uniarticular peduncle bears a plumous seta representing the inner branch and a stout outer branch on the distal extremity, and no trace of muscle is noticeable. The outer branch is provided with four simple setae on its distal end.

II Stage (Pl. XI, Fig. 109). The peduncle now consists of three segments; and a series of plumous setae are borne on the inner margin and on its terminal margin. Some simple setae are at or near the articulation between these segments on the outer margin. The proximal peduncular segment is longer than the succeeding two segments, is provided with a spinule near the middle of the inner margin, and is externally expanded near the base; this external expansion will develop to a stylocerite in the later stages. The "antennular lobe" of Gurney is noticeable in this stage. The inner branch, which was represented by a plumous seta in the preceding stage, is now quite thick at the base, while the outer branch is provided with some numbers of aesthetes at the distal extremity. The muscles are now noticeable in the peduncular segments; viz., in each of the peduncular segments there are two longitudinal muscles, while in the proximal segment two more oblique ones are noticeable besides these.

III Stage (Pl. XI, Fig. 110). In the proximal peduncular segment the external expansion near the base of the outer margin is now terminally curved forwards and it takes the form of the rudimentary stylocerite. Both of the terminal two branches are elongated, but are not articulated. The muscles are similar to those of the preceding stage.

IV Stage (Pl. XI, Fig. 111). The setae on the margin of the peduncular segments are increased in number. The outer branch now consists of two articles, while the inner is still uniarticular; the latter terminates in two setae, a long and a short.

V Stage (Pl. XI, Fig. 112). Each of the flagella consists of three articles, though the outer is longer than the inner. In the former, the terminal article is longer than any of the proximal two, and is provided with two aesthetes on its inner margin, while in the latter the terminal article is very short and is provided with three slender setae on the distal extremity. In the proximal peduncular segment a rudimentary statocyst is noticeable at the base.

VI Stage (Pl. X, Fig. 113). The terminal article of the inner flagel-

lum elongates and is about as long as the penultimate, which is furnished with a small seta near the extremity; while in the outer flagellum the terminal article is a little folded where the aesthetes are besetted. The stylocerite as well as the muscles is now well developed.

VII Stage (Pl. XI, Fig. 114). Both of the flagella consist of four articles; on the outer flagellum the branching appendix is furnished and arises from the third article, and the terminal article of the preceding stage is now divided into two articles. Two aesthetes are besetted on the distal extremity of the branching appendix.

VIII Stage (Pl. XI, Fig. 115). The inner flagellum consists of five articles, while the outer is not only elongated at its terminal article, but the proximal is divided into two articles, so that now it consists of five articles. The branching appendix of this flagellum seems to be retracted in position and it arises from the third article.

IX Stage (Pl. XI, Fig. 116). The inner branch consists of about ten articles and the outer of eight. The branching appendix of the latter branch arising from the third article is divided into two articles and is terminally provided with two aesthetes as was that of the preceding stage.

X Stage (Pl. XI, Fig. 117). The peduncular segments are provided with more numbers of marginal plumous setae on the inner margin. The articles of the inner flagellum are eleven or twelve in number, while those of the outer are thirteen, and its branching appendix arising from the third article consists of three articles and the terminal aesthetes seen in the preceding stage now transform to small setae.

2. The Second Antenna.

I Stage (Pl. XI, Fig. 118). It consists of the protopodite and two branches, the exopodite and the endopodite. The protopodite is rather long and is armed with a minute spinule at the distal extremity of the inner margin. The exopodite is foliaceous in form and is a little plicated near the distal extremity. At this plication the outer margin is deeply notched. This notch is semi-circular in form and is guarded with a small simple seta in front and with a longer plumous seta behind; while on the anterior and inner margin nine plumous setae are provided. The inner branch or endopodite, which is rod-like in form and reaches almost to two-thirds of the outer branch, is armed with a long plumons seta and a small spinule at the distal extremity. Two muscles are noticeable in the protopodite

attached to the base of the exopodite; and no other muscle is developed in this stage.

II Stage (Pl. XI, Fig. 119). The rounded notch of the outer branch seen in the preceding stage, now disappears, and the plumous seta behind the notch transforms to the outer tooth of the scaphocerite. The margin, which has been notched in the preceding stage, is now more or less produced and a seta is provided on it; the marginal setae are now about twenty two in number. The inner branch, which is nearly as long as or somewhat longer than two-thirds the length of the outer, consists of three articles and is devoid of the terminal plumous seta. In the articles of the endopodite the terminal one is very long and is provided with some numbers of small setae. The muscles are quite well developed in this stage; in the protopodite there are two muscles attaching to the base of the endopodite besides the two seen in the preceding stage. In the proximal segment of the endopodite there are two rudimentary ones and in the exopodite two muscles are noticeable; the latter are also very small.

III Stage (Pl. XI, Fig. 120). The terminal tooth of the outer margin of the scaphocerite is now increased in size, and the scaphocerite is ordinary in the outer feature. The inner branch, which almost reaches to the distal end of the outer, consists of four articles, in which the proximal two are short as in the preceding stage and the terminal is very short, while the penultimate is the longest of all and very finely divided into four articles. The muscles are similar to those of the preceding stage.

IV–VII Stages. In the fourth stage (Pl. XI, Fig. 121), the inner branch is a little longer than the scaphocerite and consists of seven articles, in which the proximal is stout and is armed with a seta near the distal end. In the fifth stage (Pl. XI, Fig. 122), however, the flagellum exceeds the extremity of the outer scale by about one-third of its length and consists of about twelve articles, some of which are provided with one or two small setae. The flagellum of the next stage (Pl. XI, Fig. 123) exceeds the extremity of the outer scale by about two-fifths of its length and is divided into about fifteen articles. In the seventh stage it is longer than twice the length of the scaphocerite. In these stage, the marginal setae of the scaphocerite are successively increased in number.

VIII Stage (Pl. XI, Fig. 124). The inner branch is more than two and a half times the length of the scaphocerite; and the proximal two articles are well developed and form the peduncle of the inner branch; the latter are now movable, as in these two peduncular articles muscles are developed. The marginal setae of the scaphocerite are more than thirty-five in number.

IX–X Stages. In the ninth stage the inner branch is three and a half times as long as the scaphocerite; and in the tenth stage it is almost four times the length of the latter.

3. The Mandible.

I Stage (Pl. XI, Fig. 125). It is simple and rather weak, and consists of a more or less bending stalk and a cutting portion. On the inner margin of the latter, there are seven teeth, of which two near the anterior corner are longer than the others and are terminally obtusely pointed, while the remaining five teeth are rather sharply pointed. Of these five teeth the posterior two are very small in size. Besides these teeth on the margin, there are several tubercular teeth on the inner surface.

II Stage (Pl. XI, Fig. 126). The posterior part of the inner margin where two small teeth have been besetted in the preceding stage, is now much developed and forms a posterior lobe in company with a succeeding proximal tooth; while on the other hand the anterior corner, where two large teeth have been provided in the preceding stage, produces inwards, thus the inner margin is divided into two lobes.

III Stage (Pl. XI, Fig. 127). In this stage one more tooth develops on the deeply notched margin between the anterior and the posterior lobe, and the other three teeth on this notch are somewhat more slender in form than those in the preceding stage. One more small tooth arises on the anterior lobe.

IV Stage (Pl. XI, Fig. 128). One more tooth comes out on the anterior lobe, which now bears four teeth; among these teeth one is very broad. The three acutely pointed teeth on the notch stand close together and are now spinular in shape.

V Stage (Pl. XI, Fig. 129). The broad tooth on the anterior lobe is now flattened and terminally truncated. On the notch between the anterior and the posterior lobe there are some increased numbers of setae and a rather stout tooth,—the latter is terminally rounded.

VI Stage (Pl. XI, Fig. 130). The anterior lobe seems to be somewhat folded inwards, and a tooth on this lobe is now a short distance retracted in position; and some numbers of sharply pointed setae on the notch are regularly arranged in a series.

VII Stage (Pl. XI, Fig. 131). One tooth on the anterior lobe is degenerated, while on the posterior lobe some numbers of small setae are provided on the margin. Between these two lobes, the margins of the

notch and the ridge, on which sharply pointed setae are arranged, are now distinctly separated; and the anterior half of the former is more *excavated* than the margin of the latter, while on the posterior half they are almost parallel.

VIII Stage (Pl. XI, Fig. 132). The ridge on which the setae are arranged is transformed to a ridge of the molar process and at the same time the setae are decreased in size. The inner margin of this appendage is now distinctly divided into two processes,—the incisor and the molar. As has been described above, the inner margin was divided into two lobes, an anterior and a posterior, in the early stages, but this distinction does not coincide with that between these incisor and molar processes. The palp is not noticeable in this stage.

IX Stage (Pl. XI, Fig. 133). In the molar process, the ridge on which more or less abbreviated setae are provided is transformed to an inner ridge of the grinding surface. The palp comes out for the first time in this stage as a bud, which is articulated at the base of the incisor process. At the base of the palp two muscular bundles are noticeable.

X Stage (Pl. XI, Fig. 134). One more ridge is developed on the grinding surface of the molar process. The palp is a little increased in size and now distinctly consists of two articles. Besides two muscles at the base of the palp one more came out at the inner side of the inner one but I can not ascertain its function.

4. The First Maxilla.

I Stage (Pl. XI, Fig. 135). It consists of three lobes. The anterior lobe is distinctly separated from the other two, and terminates in a simple and rather stout seta. The remaining two lobes, however, are closely in contact with each other, and each is armed with four setae on the inner margin, but in the posterior lobe one more slender seta is provided on the posterior margin. A muscle is noticeable in the middle lobe and is attached to the base of the anterior lobe.

II Stage (Pl. XII, Fig. 136). The anterior lobe is tuberculated near the base of the terminal seta on the outer margin. In the remaining two lobes the upper is now provided with seven setae on the inner margin, while in the lower the setae are still similar to those of the preceding stage.

III–VI Stages. In the third stage (Pl. XII, Fig. 137), the tubercle near the extremity of the anterior lobe increasing in size grows up forwards to the distal extremity of this lobe, and the seta now seems to be besetted

near the distal end of the inner margin. In the succeeding three stages (Pl. XII, Figs. 138 and 139), any characteristic changes in feature are not noticeable, though the setae on the inner margin of the basal two lobes are somewhat increased in number in the later stages.

VII Stage (Pl. XII, Fig. 140). The anterior lobe is somewhat folded inwards at the distal extremity and is now distally truncated; the seta of this lobe seen in the preceding stage is on the inner corner of this distal margin. The other two lobes are each provided with six setae, besides these one more seta is provided on each lobe, on the anterior margin of the anterior lobe and on the posterior margin of the posterior.

VIII Stage (Pl. XII, Fig. 141). The anterior lobe is distally emarginated; and its terminal half is strongly curved inwards so that the terminal two processes are now anterior and posterior in relation. The seta of this lobe is on the posterior process and is much decreased in length. The other two lobes are well developed and are providod with more numbers of setae on their inner margins and with one or two slender setae on the posterior margin of each lobe. The muscle, which has been seen at the base of the anterior lobe in the preceding stages, extends in this lobe.

IX Stage (Pl. XII, Fig. 142.) The anterior lobe is similar in feature to that of the preceding stage. The other two lobes are distinctly separated in position and increased numbers of setae are provided on the inner margins.

X Stage (Pl. XII, Fig. 143). The marginal setae on the posterior two lobes are increased in number and are strong. Two or three muscular bundles are developed in the proximal lobe of this appendage.

5. The Second Maxilla.

I Stage (Pl. XII, Fig. 144). It consists of five lobes, of which the outer is foliaceous in shape and is the largest of all. About twelve plumous setae are on the margin of this lobe, and the posterior one in these setae is the longest and is directed posteriorly. Of the remaining four lobes the anterior is distally truncated, while the others are terminally rounded. Three or four setae are provided on the terminal or inner margin of each of these lobes. A muscle arising near the centre of this appendage is terminally expanded forwards and outwards into the outer lobe; besides this there are two muscular bundles at the base of this appendage.

II–III Stages. In the second stage (Pl. XII, Fig. 145), it is similar to that of the preceding stage, but the outer foliaceous plate is developed and

the marginal setae are about twenty in number. The muscle in this outer lobe is divided into two bundles. In a similar feature it passes through the third stage, in which, however, the marginal setae of the outer foliaceous plate are about twenty four in number.

IV Stage (Pl. XII, Fig. 146). On the margin of the outer foliaceous plate there are about twenty eight plumous setae, of which the posterior two are long and stout. The remaining four lobes are similar to those of the preceding stage. Besides the muscles seen in the preceding stage, there are three small ones, of which one is in front of those in the outer plate, another is at the articulation between the outer plate and the inner lobes, and the remaining one is in the inner anterior lobe.

V Stage (Pl. XII, Fig. 147). The marginal plumous setae of the outer foliaceous plate are about thirty two in number, and of these setae the posteriorly directed two are still longer and stouter than the others. Each of the inner four lobes is furnished with more numbers of setae; i. e., the anterior lobe is provided with two at the outer corner and with three at the inner corner of the truncated margin, and each of the remaining three lobes is provided with four setae on the inner margin. The muscle in the anterior lobe is degenerated, while the outer two muscles, which were rudimentary in the preceding stage, are now well developed and extended backwards.

VI Stage (Pl. XII, Fig. 148). The outer foliaceous plate is armed with about thirty six marginal setae, in which the posterior three are stout. Of the inner four lobes the anterior is provided with three setae, one on the outer and two on the inner corner; therefore they are similar to those in the stages before the fourth. The muscle, which arises near the centre of this appendage, is extended backwards, is somewhat retracted in position and transformed to an inner basal one.

VII Stage (Pl. XII, Fig. 149). The marginal setae of the outer plate are about forty two in number, and in these setae the posterior five are stout. The anterior of the inner lobes is more or less decreased in length, but a little enlarged to a tubercle at the distal inner corner, where two setae are provided. The posterior lobe is a little retracted in position compared to the succeeding two lobes.

VIII Stage (Pl. XII, Fig. 150). The outer foliaceous plate is armed with more than forty-five marginal setae and some of the posterior ones are long and stout. Of the inner four lobes the anterior one, which is represented by an obtusely pointed lobe, is devoid of setae, while the two lobes behind this are much developed and are provided with more numbers

of setae on their inner margins. The basal lobe is much retracted in position and is provided with a very small seta near the anterior end of the inner margin.

IX Stage (Pl. XII, Fig. 151). The marginal setae of the outer foliaceous plate are about fifty in number. Of the inner four lobes, the proximal is still retracted in position while the succeeding two lobes are more produced inwards. The muscle in the anterior lobe appears once more in this stage, and two or three are at the base of the inner lobes.

X Stage (Pl. XII, Fig. 152). The marginal setae of the outer plate are now about sixty eight in number, and some of the posterior ones are not so stout nor so long as those in the preceding stage. The inner basal lobe is more retracted in position and its margin is devoid of seta. It thus attains to the feature of the adult.

6. The First Maxilliped.

I Stage (Pl. XII, Fig. 153). On the basal segment two branches articulate, the exopodite and the endopodite. The basal segment or protopodite is rather stout and is armed with four or five small setae on the inner margin. The exopodite, which terminates in two long setae and is provided with a long seta on each side closely attached to the base of the terminal one, is a little more than twice the length of the endopodite. The latter is slender in form, apparently four-segmented, and the basal segment is the shortest of all, while the third or penultimate segment is the longest. The terminal segment is provided with three setae on the extremity and with one more small one on the outer margin. On the inner margin of the third segment there is a seta near the articulation with the terminal segment. Two muscles are noticeable in the exopodite, at the base of which two more muscles are attached. There is no muscle attached to or in the endopodite.

II–V Stages. In the second stage (Pl. XII, Fig. 154), it is similar in feature to that of the preceding stage, but the endopodite now consists of three segments, in which the proximal two are subequal in length and each is longer than the terminal one. In the succeeding stages it is similar in feature, but in the third stage (Pl. XII, Fig. 155) two small muscles are attached to the base of the endopodite though in the next stage these muscles are not noticeable. In the fourth stage (Pl. XII, Fig. 156), the protopodite is indistinctly divided into two segments; and the terminal segment of the endopodite bears four setae on its distal extremity; in the

next stage (Pl. XI, Fig. 157), however, the proximal segment of the protopodite is provided with a tubercle on the outer margin.

VI Stage (Pl. XII, Fig. 158). The protopodite is now well developed and the tubercle on its outer margin is somewhat increased in size, while on the inner margin there are five or six setae. The exopodite is a little dilated outwards at the proximal part and two small setae are provided on the margin.

VII Stage (Pl. XII, Fig. 159). The protopodite is dilated inwards; its proximal segment bears a seta and the terminal more than ten setae, on their inner margins. The tubercle on the outer margin is developed and two-lobed. The exopodite is more dilated outwards near the base and about six setae are provided on the margin of this dilation. The two muscles at the base of the exopodite are somewhat degenerated.

VIII–IX Stages. In the eighth stage (Pl. XII, Fig. 160), the proximal segment of the protopodite is more developed and is provided with four setae on its inner margin, and the terminal segment has many setae. The outer tubercle or the rudiment of the epipodite is now distinctly two-lobed. The endopodite being much degenerated is not articulated and is devoid of setae. The outer expansion at the base of the exopodite is now remarkable and more than eight setae are provided on the margin. Of the two muscles at the base of the exopodite, the posterior one only is noticeable as a rudiment. In the next stage (Pl. XII, Fig. 161), it is similar in feature, but the proximal segment of the protopodite is a little more developed and bears six setae on the margin; at the same time, the anterior segment is also developed, the marginal setae on the outer expansion at the base of the exopodite are about twelve in number, while the muscles are generally more degenerated.

X Stage (Pl. XII, Fig. 162). The proximal segment of the protopodite is much developed and is armed with about ten rather stout setae on the inner margin. On the outer expansion at the base of the exopodite there are about twelve marginal setae, while on the distal part of the exopodite there are two or three marginal setae besides the four terminal long ones.

7. The Second Maxilliped.

I Stage (Pl. XIII, Fig. 163). The basal segment or protopodite bears two branches, the exopodite and the endopodite. The protopodite is provided with three or four setae on the inner margin. The exopodite is a

little longer than twice the length of the endopodite, bears ten plumous setae, of which four are closely in contact with each other at the extremity and the remaining six are on the lateral margin, three on each side. The endopodite consists of four segments, of which the proximal is very short and is provided with a seta on the inner margin, while the next is longer than any of the others and is devoid of setae. The third or penultimate segment is somewhat shorter than the former, is armed with two setae on the inner margin; and the terminal segment has three setae at the distal end. The muscles are more developed than those of the preceding pair of appendages; in the protopodite there are four muscles:—two are attached to the base of the exopodite and the other two to that of the endopodite. Two longitudinal muscles are in the exopodite; and in each of the second and the third segment of the endopodite there are two muscles.

II–IV Stages. In the second stage (Pl. XIII, Fig. 164), the exopodite bears four setae on each margin besides the terminal four, and at the distal extremity of the endopodite there are four setae and one more small seta is provided near the base of the terminal segment on the outer margin. In the next stage (Pl. XIII, Fig. 165), the proximal segment of the endopodite bears a small seta on the inner margin, and the third segment now bears a seta on the inner margin and another on the distal margin between the inner and the outer margins. In the fourth stage (Pl. XIII, Fig. 166), among four setae on the terminal segment of the endopodite the terminal one is much stouter and longer than the remaining three.

V Stage (Pl. XIII, Fig. 167). In the endopodite the third segment in the preceding stage is apparently divided into two segments, so that it is now five-segmented, but this articulation indicates no significance as to the arrangement of the muscles. The terminal segment now bears five setae, one of which is very strong.

VI–VII Stages. In the sixth stage (Pl. XIII, Fig. 168), the protopodite is indistinctly divided into two segments, and the exopodite bears three setae on each margin besides the terminal four. In the next stage (Pl. XIII, Fig. 169) the protopodite is a little dilated on the outer margin, and the terminal segment of the endopodite bears two setae on the outer margin besides the terminal four. The muscles in the exopodite are more or less degenerated.

VIII Stage (Pl. XIII, Fig. 170). A striking modification occurs in this stage. Each segment of the protopodite bears a seta on the inner margin, and the outer margin is much inflated and indistinctly two-lobed. This is the rudiment of the podobranchia. The setae of the exopodite

are more or less degenerated; four setae near the distal end are short in length and one or two rudimentary ones are on each margin. The endo-podite consisting of five segments is rather stout in form; especially the erminal two segments are very broad and are provided with numerous etae on the margins. The muscles are generally well developed, though hose of the exopodite are somewhat degenerated. Of the two muscles at he base of the endopodite, one is still in the same place, while the other s in the proximal segment of the endopodite and is attached to the second egment. The articulation between the penultimate and the antepenultimate egments is apparently distinct, but according to the arrangements of the nuscles this articulation is still insignificant.

IX Stage (Pl. XIII, Fig. 171). The proximal segment of the pro-opodite is well developed and bears three setae on the inner margin, while he terminal segment is fused with the proximal segment of the endopodite. The rudimentary podobranchia is terminally three-lobed, besides which he outer margin is dilated so as to form the epipodite. On the margins f the terminal two segments of the endopodite there are increased numbers f setae. Some muscles in the proximal part of this appendage are more r less degenerated.

X Stage (Pl. XIII, Fig. 172). The proximal segment of the proto-odite is more developed; and the next segment, which has its origin in he terminal segment of the protopodite and the proximal segment of the ndopodite, bears the exopodite and the podobranchial plume. The epipodite s developed a little behind the podobranchia. The setae on the terminal wo segments of the endopodite as well as those on the exopodite are ncreased in number.

8. The Third Maxilliped.

I Stage (Pl. XIII, Fig. 173). The protopodite bears two branches, he exopodite and the endopodite. The protopodite bears three setae on the nner margin. The exopodite bears twelve setae, of which four are near he extremity closely in contact with each other and the remaining ones re on the margins, four on each side. The endopodite, which is two-thirds s long as the former, consists of five segments. In these segments the econd is the longest of all, and the penultimate is shorter than the former, ut is distinctly longer than any of remaining three; the latter are sub-qual in length. On the inner margin a seta is provided on each of the rst, the second, and the fourth segments. The terminal segment bears

three setae near the extremity, a stout one and two slender, and one more small seta near the base of the outer margin. The muscles in the protopodite and the exopodite are similarly developed to those of the preceding pair. But those of the endopodite are somewhat different; there are two muscles in each of the second, the third, and the fourth segments, while no muscle is noticeable in the proximal segment.

II Stage (Pl. XIII, Fig. 174). The endopodite is elongated; on its inner margin the second segment is provided with two setae, the third with one, and the fourth with two; and the latter has one more on the outer margin. The terminal segment is provided with one more small seta near the extremity.

III Stage (Pl. XIII, Fig. 175). The third segment of the endopodite now bears one more seta on the terminal margin between the outer and the inner sides. The exopodite is provided with one more seta on each margin.

IV–V Stages. In the fourth stage (Pl. XIII, Fig. 176) the protopodite is indistinctly divided into two segments. On the outer margin of the endopodite the second and the third segments as well as the fourth are each provided with a seta near the distal end. In the next stage (Pl. XIII, Fig. 177) it is much similar in feature, but the terminal segment of the endopodite bears four small setae near the extremity besides a strong terminal one.

VI Stage (Pl. XIII, Fig. 178). In the endopodite the third segment is provided with four setae, of which three are on the distal margin, one on each side of the outer and the inner, and another is between both the sides. One more seta of this segment is near the middle of the inner margin. The penultimate segment bears four setae at the distal extremity two on each side.

VII Stage (Pl. XIII, Fig. 179). The endopodite is now a little longer than five-sixths the length of the exopodite; and its penultimate segment is provided with one more seta a little behind the extremity besides the terminal four. The muscles attaching to the base of the exopodite are somewhat degenerated.

VIII Stage (Pl. XIII, Fig. 180). The protopodite is indistinctly divided into two segments. The exopodite is much degenerated; i. e., not only is it decreased in length hardly reaching to the middle of the antepenultimate segment of the endopodite, but it bears only two rudimentary setae on the extremity. In the endopodite the proximal two segments are almost fused, and the terminal segment is now very short and bears two

setae on the distal end. On the inner margin all segments but the terminal one are provided with some increased numbers of setae. The muscles in the exopodite and in the protopodite as well as those in the penultimate segment of the endopodite are more or less degenerated.

IX Stage (Pl. XIII, Fig. 181). The exopodite, which does not reach to the distal end of the second segment of the endopodite, bears four long setae on its distal extremity. The terminal segment of the endopodite is decreased in size, and the articulation with the preceding segment is now indistinct. More increased numbers of setae are provided on each segment of the endopodite. On the outer margin of the protopodite a rudimentary tubercular lobe comes out.

X Stage (Pl. XIII, Fig. 182). In the endopodite the articulation between the terminal two segments disappears and on the other hand that between the proximal two segments is also indistinct, so that the endopodite now consists of three segments. The exopodite is provided with ten setae, of which four are on the distal extremity as well as those in the early stages. The rudimentary lobe which was seen in the preceding stage near the outer margin of the protopodite is now well developed and is transformed to the arthrobranchia. The muscles in the proximal part of this appendage are well developed.

9. The First Pereiopod.

I Stage. It is represented by a terminally two-branched sac.

II Stage (Pl. XIII, Fig 183). It is now distinctly segmented. The protopodite bears two branches of which the outer or exopodite is a little longer than the inner or endopodite. The protopodite is provided with three setae on the inner margin. The exopodite bears twelve setae, of which four are near the extremity and the remaining ones are on the margin, four on each side. The endopodite consists of five segments, of which the second is the longest; the fourth is somewhat shorter than the former, but is distinctly longer than any of the others. The proximal is the shortest of all. On the inner margin of the endopodite each of the first and the third segments is provided with a seta, and the fourth with two setae; while on the outer margin a seta is provided on each of the third and the fourth segments. The terminal segment bears two setae, a stout and a slender, on its distal end. The muscles are distributed similarly to those of the preceding pair in the corresponding stage.

III Stage (Pl. XIII, Fig. 184). The endopodite is a little shorter

than two-thirds the length of the exopodite, and the latter is provided with five setae on each margin besides the terminal four. The penultimate segment of the endopodite is somewhat dilated at the distal inner corner, where two setae have been provided in the preceding stage, but now one of these setae is changed in position somewhat near to the outer margin.

IV Stage (Pl. XIII, Fig. 185). The exopodite is provided with fourteen setae, of which four are near the distal end and the others are on the margins, five on each side. In the endopodite the penultimate segment is distinctly rounded at the distal inner corner, where now two setae are provided; the terminal segment is stout, but is somewhat decreased in length.

V–VII Stages. In the fifth stage (Pl. XIII, Fig. 186), the process at the distal inner corner of the penultimate segment of the endopodite develops into the immovable finger of the chela. The setae are distributed as were those of the preceding stage, but the third segment is provided with one more seta on its distal margin in the middle of the inner and the outer margins. In a similar feature it passes through the succeeding two stages (Pl. XIII, Figs. 187 and 188), but in the seventh stage the setae of the endopodite are generally decreased in length and two rudimentary muscles are noticeable in the proximal segment of the endopodite.

VIII Stage (Pl. XIII, Fig. 189). The protopodite is divided into two segments, each of which is provided with two short setae on the inner margin. The exopodite is much degenerated and is devoid of setae, while in the endopodite, which is now well developed, the proximal segment is armed with three setae, two on the inner and the third on the outer margin. The next or second segment is provided with a seta near the middle of each margin and with one more at the distal end of the inner margin. The third segment or the carpos is now about as long as, or somewhat longer than, the preceding segment and bears two setae at the distal end of the inner margin. The finger of the chela is nearly as long as the palm, and its distal extremity is pointed and slightly curved inwards. The setae are born in some numbers of groups on both of the fingers. The muscles are generally well developed, but those of the exopodite are more or less degenerated.

IX Stage (Pl. XIII, Fig. 190). The exopodite is now entirely degenerated and the distal segment of the protopodite or the basis has its outer margin smooth. The finger of the chela is a little shorter than the palm. The muscles attached to the exopodite are all degenerated.

X Stage (Pl. XIII, Fig. 191). The basis is decreased in length

especially at the outer margin, so that the posterior process of the succeeding segment or the ischium almost touches the coxal joint; and three or four rather strong setae are provided on the inner margin of the basis. The carpos is elongated and is somewhat longer than the merus, these two segments have been subequal in length in the preceding two or three stages.

10. The Second Pereiopod.

It is similar in feature as well as in mode of the development to the preceding pair, but it is somewhat smaller in size than the latter in the second stage (Pl. XIV, Fig. 192); in the third stage (Pl. XIV, Fig. 193), however, it is almost equal in size in the corresponding stage. In the early stages before the fourth (Pl. XIV, Fig. 194), the exopodite bears twelve setae; while in the succeeding three stages, the fifth, the sixth, and the seventh, it bears fourteen setae (Pl. XIV, Figs. 195, 196, 197). In the eighth stage (Pl. XIV, Fig. 198), the exopodite is more degenerated than that of the preceding pair in the corresponding stage and in the muscles of the exopodite a rudimentary one is noticeable at its base, while the remaining ones are all degenerated. In the ninth stage (Pl. XIV, Fig. 199), the carpos is elongated and is somewhat longer than the merus; and in the next stage (Pl. XIV, Fig. 200), the former is about one and one-third the length of the latter, and the setae on the inner margin ot the basis joint are somewhat slender.

11. The Third Pereiopod.

In the second stage it is still represented by a terminally two-branched sac, and the muscle is not developed as shown in Pl. XIV, Fig. 201.

III Stage (Pl. XIV, Fig. 202). It is smaller in size than that of the preceding pair in the corresponding stage. The protopodite is devoid of setae; while the exopodite is provided with ten setae, of which four are near the distal end and the remaining six are on the margin, three on each side. The endopodite, which is a little shorter than the exopodite, apparently consists of four segments, as the proximal two segments are fused into one segment, but a small seta is provided on the inner margin, where the segment is supposed to be articulated. The penultimate segment of the endopodite is provided with two setae at the distal end of the inner margin, while the terminal segment bears two setae, a stout and a slender. The muscles are distributed similarly to those of the preceding pairs.

IV Stage (Pl. XIV, Fig. 203). The protopodite is now provided with three setae on the inner margin. The endopodite is now five-segmented, and its antepenultimate segment is provided with a seta on each margin of the outer and the inner. The next or penultimate segment bears one more seta at the distal end of the outer margin, while the terminal is as that in the preceding stage.

V–VI Stages. In the fifth stage (Pl. XIV, Fig. 204), the penultimate segment of the endopodite is provided with one more seta on the outer margin, so that it bears now two setae on each margin near the distal end. Each of the second and the third segments is also provided with a seta on the outer margin. In the sixth stage (Pl. XIV, Fig. 205), the third segment bears one more seta at the distal end; the exopodite is now provided with one more seta on each margin.

VII Stage (Pl. XIV, Fig. 206). The protopodite is indistinctly divided into two segments. The endopodite elongates and is somewhat longer than the exopodite. The muscles in the latter are more or less degenerated.

VIII Stage (Pl. XIV, Fig. 207). The protopodite is now distinctly divided into two segments, of which the distal one is shorter than that of the preceding pair in the corresponding stage. The exopodite is degenerated. Somewhat increased numbers of setae are provided on the terminal two segments of the endopodite, while those of the two or three proximal segments are rather decreased in number. The muscles are well developed except those of the exopodite. In the coxal segment there are three muscles and in the next segment as well as in the proximal segment of the endopodite a muscular bundle is noticeable.

IX Stage (Pl. XIV, Fig. 208). The exopodite is more degenerated, but it is still noticeable as a minute tubercle at the outer corner of the terminal segment of the protopodite, where the latter is very short and the basal segment of the protopodite and the proximal of the endopodite are quite close to each other. The said proximal segment of the endopodite is provided with three setae on the inner margin and two on the outer; the next segment has two setae on the inner margin and has two or three on the margin where the next segment is articulated, and the third segment has three setae, of which two are on the inner margin and another one is on the outer. The penultimate segment bears two setae on the inner and one on the outer margin besides some numbers of terminal setae; while the setae on the terminal segment are distributed similarly to those of the preceding stage. The muscles in the distal segment of the protopodite and in the proximal segment of the endopodite are well developed.

X Stage (Pl. XIV, Fig. 209). The exopodite, which has been represented by a minute tubercle in the preceding stage, now entirely disappears. In the endopodite the proximal segment, which is now the third segment of this appendage, is provided with two setae on each margin; and the next segment with two on the inner and one on the outer margin besides the terminal two setae, while in the third segment the setae are similar to those of the preceding stage. The setae on the penultimate segment increase in number; on the inner margin there are three spinules, each of which is guarded by two or three small setae; on the outer margin, however, the setae are apparently irregularly arranged.

12. The Fourth Pereiopod.

III Stage. In the third stage, it is still represented by a terminally two-branched sac.

IV Stage (Pl. XIV, Fig. 210). It is now distinctly segmented, but smaller in size than the preceding pair in the corresponding stage. The protopodite, which is devoid of setae, distally bears the exopodite and the endopodite; in these two branches the former is as long as three-fourths of the latter and is provided with five setae, of which four are near the distal end and the remaining one is on the outer margin a little behind the extremity. The endopodite consists of five segments, of which the first is provided with a seta on the inner margin while the second is devoid of setae. The third segment bears a seta on the outer margin, the penultimate is provided with two setae at the distal end of the inner margin, and the terminal one has one strong and one small seta at the distal end. The muscles are well developed as were those of the preceding pair of this stage and a rudimentary one is noticeable in the proximal segment of the endopodite.

V Stage (Pl. XIV, Fig. 211). The endopodite is now larger in size than that in the preceding stage. The protopodite is provided with two small setae on the inner margin. The exopodite is a little less than two-thirds the length of the endopodite and is provided with eight setae, of which four are near the distal end and the remaining four are on the margin, two on each side. In the segments of the endopodite, each of the proximal two segments is provided with a seta near the distal end, in the first on the inner, and in the second on the outer, margin. The third segment is distally provided with two setae, one on each margin; and the penultimate segment has three, one on the outer and two on the inner

margin. The muscle in the proximal segment of the endopodite is not noticeable in this stage.

VI Stage (Pl. XIV, Fig. 212). The endopodite is elongated and is now a little shorter than twice the length of the exopodite. In nearly all other respects it is similar to that of the preceding stage, but the penultimate segment of the endopodite is distally provided with two setae on each margin.

VII Stage (Pl. XIV, Fig. 213). The protopodite is indistinctly divided into two segments; the endopodite is now distinctly more than twice the length of the exopodite; the latter is now provided with ten setae, the marginal six and the terminal four. The muscle in the proximal segment of the endopodite once more appears. In other respects it is similar to that of the preceding stage.

VIII Stage (Pl. XIV, Fig. 214). It is somewhat decreased in length. The protopodite is divided into two segments, each of which is provided with a seta :—on the outer in the proximal and on the inner margin in the distal segment. The exopodite is considerably degenerated. In the endopodite, the proximal two segments are devoid of setae, while each of the succeeding two segments is provided with a seta on the distal extremity of each margin. The terminal strong seta of the terminal segment decreasing in length transforms to an elongated point of the dactylos and is devoid of any other setae.

IX Stage (Pl. XIV, Fig. 215). It is now more or less elongated. The exopodite disappears. The two segments of the protopodite are devoid of setae; the next or proximal segment of the endopodite is provided with a small seta at the distal end of the outer margin; while the next segment bears three setae, two on the inner and one on the outer margin, besides some numbers of terminal ones. The next segment is distally provided with three setae of which two are on the inner margin. The penultimate segment bears two on the inner and three on the outer margin, and the terminal segment has one more small seta on the outer margin. In the stages after this the setae on the terminal segment gradually increase in number.

13. The Fifth Pereiopod.

I Stage. It is imbedded in the medial line of the ventral surface between the rudiments of the anterior two pairs of pereiopods.

II Stage (Pl. XIV, Fig. 216). It is now free from the ventral

surface of the thorax and indistinctly divided into six segments, but is devoid of setae. The muscles are rudimentary, but noticeable in each segment.

III Stage (Pl. XIV, Fig. 217). It is now well developed, extraordinarily long and stout, and uniramous. It is still divided into six segments, of which the third is about as long as the fifth, and the remaining four are distinctly shorter than these two. The proximal four segments are devoid of setae, while the penultimate is provided with four conspicuous setae, two longer and two shorter, on the inner margin. This margin is ordinarily faced backwards, but in this appendage the terminal segments are inverted in position, and when the appendage is directed forwards the inner margin is faced to the ventral surface of the cephalothorax. The terminal segment is terminally curved to the surface of the cephalothorax and two stout setae are provided on it, one is stronger than the other. This stronger seta is on the distal end of this segment and the other is on the inner margin. In each segment except the terminal there are two muscles, which attach to the base of the succeeding segment.

IV Stage (Pl. XIV, Fig. 218). It consists of seven segments, as the third segment in the preceding stage is now divided into two segments, the proximal of these two segments is provided with a small seta at the distal end of the inner margin. The penultimate segment is now provided with five setae, of which two are longer than the others. The terminal segment is similar in feature to that of the preceding stage, but is somewhat more elongated and more strongly curved inwards. A rudimentary muscle is noticeable in the third segment.

V Stage (Pl. XIV, Fig. 219). On the inner margin a small seta is provided on each of the third and the fifth segment, while the fourth bears a seta on the outer margin. The penultimate segment is now provided with two small setae near the distal end, one on each margin, besides the four strong setae on the inner margin. In the terminal segment the terminal strong seta decreases in length and at its base there is a small seta on the outer margin, while the strong one on the inner margin is still similar in feature to that of the preceding stage.

VI Stage (Pl. XIV, Fig. 220). One more small seta is provided on the inner margin of the fourth segment. On the penultimate segment there are now five strong and two small setae; the latter are near the distal extremity, one on each margin, while the former are all on the inner margin as seen in the preceding stage.

VII Stage (Pl. XIV, Fig. 221). The penultimate segment is pro-

vided with six strong setae on the inner margin besides the terminal two small setae; and in the terminal segment the seta on the inner margin is decreased in length and the small seta at the base of the terminal strong seta entirely disappears.

VIII Stage (Pl. XIV, Fig. 222). Modifications in many respects occur in this stage. It has been inverted in position compared to the preceding pairs, but it is now ordinary and decreased in length, still however, it is considerably longer than the preceding legs. The third segment is provided with two setae on the inner margin, the fourth with two on the outer and one on the inner margin besides the terminal two or three setae, and the fifth has two setae, one on each margin. In the penultimate segment, however, the long setae which were seen in the preceding stage on the inner margin are now much decreased in length and are spinular in shape. On the outer margin there are three or four setae near the distal extremity. The terminal segment terminating in a point is devoid of setae.

IX–X Stages. *In the ninth stage* (Pl. XIV, *Fig.* 223), *it is now* somewhat stouter, but a little decreased in length. On the other hand the preceding pair of legs have increased in length so that the difference in length between these legs is not so striking as in the preceding stage. The setae or spinules on the penultimate segment is more or less increased in number. In the next stage it is not longer than the preceding pair of legs, and the muscles are well developed.

14. The First Pleopod.

At first it comes out as a small tubercle in the third stage, and in the next stage (Pl. XV, Fig. 224) it is terminally two-lobed and a rudimentary muscle is noticeable in the proximal part, but it is not differentiated from those of the succeeding four pairs in feature.

V Stage (Pl. XV, Fig. 225). It is now distinctly articulated, and the protopodite bears two branches, the exopodite and the endopodite. In these two branches, the latter is terminally rounded and a little less than one-fourth the length of the former, which is terminally somewhat tuberculated. There are two muscles in the protopodite and a rudimentary one in the exopodite.

VI Stage (Pl. XV, Fig. 226). The exopodite is much elongated and is now about one-fifth as long as the endopodite; the latter is provided with seven tubercles on the margin. The muscles are somewhat more developed than those of the preceding stage.

VII Stage (Pl. XV, Fig. 227). The endopodite is now about one-fourth the length of the exopodite; the latter is provided with nine setae on the margin, while the former is still terminally rounded and devoid of setae. The muscles in the protopodite are well developed.

VIII Stage (Pl. XV, Fig. 228). The muscles in the protopodite are developed, and apparently are more complex. The endopodite is obtusely pointed and is provided with three small setae, but no muscle is noticeable in it; while the exopodite has fourteen marginal setae and the muscle is more powerful than that in the preceding stage. It thus attains the feature of the adult, but in the later stages the marginal setae of the exopodite increase in number as shown in Pl. XV, Figs. 229 and 230.

15. THE SECOND TO FIFTH PLEOPODS.

These appendages are similar in feature, though somewhat different in size. As have been described above, they are similar at first to the preceding pair, and in the fifth stage they are differentiated from them.

V Stage (Pl. XV, Fig. 231). The appendages are distinctly articulated in this stage, as seen in the preceding pair, The endopodite is a little more than one-half the length of the exopodite, and its inner margin is slightly angulated near the middle point. The muscles are noticeable in protopodite and exopodite, but they are still rudimentary.

VI Stage (Pl. XV, Fig. 232) The endopodite is now a little more than two-thirds the length of the exopodite, and on its inner margin a rod-like appendix is indistinctly articulated. On the margins of both branches there are some numbers of small tubercles. The muscles are developed in the protopodite and there is one in each of the branches.

VII Stage (Pl. XV, Fig. 233). The endopodite, which is a little longer than two-thirds the length of the exopodite, is provided with the appendix on the inner margin, and the tubercles on the terminal margin are now somewhat prominent and are seven in number. The exopodite bears nine setae on the margin, but these setae are not so well developed as those in the succeeding stage. The muscles are well developed, but that in the endopodite is still the only one.

VIII Stage (Pl. XV, Fig. 234). The endopodite is now somewhat longer than five-sixths the length of the exopodite. The marginal setae are developed on both branches; the appendix on the inner margin of the endopodite or the stylambris is developing well, and is terminally provided with four small tubercles, which are strongly curved at their distal ends.

The muscles in the protopodite are increased in number and are apparently very complex. In each of the branches a terminally expanded muscle is noticeable, but in the endopodite besides this there is a longitudinal one, which extends along the inner margin of this branch to the base of the stylambris. Thus they attain the feature of the adult, gradually increasing the marginal setae in number in the later stages as shown in Pl. XV, Figs. 235 and 236.

16. The Sixth Pleopod.

It forms the lateral part of the rhipidura in company with the telson, and its development has been described in the place where the metamorphosis of the larval forms were described.

Summary and Discussion.

In the present paper the developments of two species of shrimps, *Paratya compressa* and *Leander paucidens* are described. In these two species, the larva hatching out from the larger egg is larger than that from the smaller egg and the former larger one is somewhat at least in a more advanced stage.[1]

In studying the metamorphosis of these larvae, a very convenient fact is that the feature of the next stage has been constructed and is lodged within the external crust of each stage just before the ecdysis is carried out.

In *Leander paucidens*, the larvae having the pelagic habit do not rest on anything in their lives, and are vertical in position, head below and tail above, and swim towards the dorsum as has been described by SOLLAUD (26) in the larvae of Palaemonid prawns[2]; while the larvae of another species, *Paratya compressa*, are horizontal in position and swim forwards. In the former species the larval pelagic life ends in the seventh stage, and a characteristic modification in feature in each of the appendages as well as a considerable reduction in size of the body occurs when it transforms to the next stage, while in the latter species the transformations in feature occur gradually at each ecdysis.

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1) The similar fact was observed by BOAS (5) in his study of two forms of the species *Palaemonetes varians*.

2) This fact was not observed in the larvae of Palaemonid prawns by BOAS (5), GURNEY (12), MAYER (20), MÜLLER (21) and WEBB (29) etc.

The *rostrum* is simple in the first stage in both the species. In *Leander paucidens* a tooth appears on the carapace in the second stage, and two more arise in the third stage; these three teeth are removed in position forwards to the upper margin of the rostrum in the eighth stage. The additional teeth appear in later stages. In *Paratya compressa,* however, the teeth of the rostrum appear in somewhat different ways. One tooth comes out in the sixth stage for the first time on the upper margin of the free part of the rostrum, and in later stages the additional teeth come out on the upper and the lower margin in a manner similar to those of *Leander paucidens* after the eighth stage.

The *supraorbital tooth* appears in the second stage in both of the species, and is still noticeable in the adult specimen in *Paratya compressa,* while in the other species it disappears in the eighth stage.

The *abdomen,* in both the species, is not only devoid of spinules, but is six-segmented at first, and the posterior margin of the laterally expanded telson is provided with fourteen setae in the first stage; while in the next stage two small setae are added between the inner pair of setae. In the later stages, the telson gradually decreases in width at the posterior extremity as compared with the width at the anterior end; and in the setae on the posterior margin, the outer two pairs are advanced in position and are transformed to the lateral marginal spinules. These facts are common to both of the species, but in *Paratya compressa* the remaining setae are subequal in length and are still noticeable in the adult on the posterior rounded margin. On the other hand, in *Leander paucidens* the outer two pairs of the remaining setae are transformed to the spinules at the outer corner and the others are mostly degenerated, but one pair transforms to a pair of plumous setae. The posterior margin is posteriorly pointed after the ninth stage.

The *first antenna* develops in a similar manner in both the species, but in *Leander paucidens* the outer flagellum is branched in the later stages; *this branch comes out from the point where two asthetes arise for the first time at the sixth stage.*

The *second antenna* is somewhat different in the two species. In *Paratya compressa* the outer branch or scaphocerite is somewhat plicated near the distal end, but is not so conspicuous as that of *Leander paucidens;* the inner branch tapering to the tip is provided near the extremity with three setae, of which the proximal is long while the others are very short and spinular in the former species, in the latter, however, it is represented by a rather long rod, on the extremity of which there are a long seta and a

nall spinule in the first stage. In the next stage the inner branch bears uch abbreviated setae near the extremity in the former species, while in ie latter it is already articulated and is much longer than that of the former.

The *mandible* is simple in feature as well as in the mode of development in the former species, but the development in the latter is somewhat different. As has been described above the inner margin of the mandible is divided into two lobes in the first stage, but these lobes are not strictly correspondents of the incisor and the molar processes. The palp comes out at first in the ninth stage, and gradually increases in size in later stages.

The *first maxilla* of the former species is in a more primitive state than that of the latter. The anterior lobe in the former, *Paratya compressa*, is provided with two setae at the distal end and with two more on the inner margin in the early stages, and these setae gradually degenerate in the later stages. In the ninth stage it is similar in feature to that of *Leander paucidens* in the early stages.

In the *second maxilla* similar facts are noticeable in the anterior lobe to that of the preceding pair, viz., the anterior lobe in *Paratya compressa* is provided with three or four setae on the inner margin, and I suppose these setae on the inner margin as well as those in the preceding pair indicate the places where the lobe is to be articulated; and in the later stages they degenerate. The outer foliaceous plate of the former species does not project posteriorly in the first stage and then it gradually develops posteriorly in the later stage, while in the latter the posteriorly projecting lobe is already represented in the first stage by a backwards directing seta.

The succeeding eight pairs of appendages are similar in feature, but in the first stage the anterior three pairs or the maxillipeds only are developed, while the remaining appendages are rudimentary. The endopodite in the maxillipeds more or less degenerates in later stages. In these thoracic appendages the protopodite is uniarticular at first, but afterwards it is divided into two segments.

In the *first maxilliped* the endopodite is distinctly segmented in the early stages in both of the species, though no muscles are noticeable in any segments; in *Paratya compressa* it consists of fonr segments in the first stage and in the fourth stage of five segments as has been described above, while on the other hand the endopodite of *Leander paucidens* also consists of four segments in the first stage, and then gradually degenerates in later stages. In the former species the proximal part of the endopodite transforms to the peculiar lobe between the protopodite and the much degen-

crated endopodite. As has been described above and shown in text-fig 9, in a specimen probably of the ninth stage the endopodite of the firs maxilliped is different in feature from the ordinary one. This abnormalit may be the "intermediate stage" of GURNEY (12); in his description o the larvae of *Palaemonetes varians* he attributed a similar form "to th bad conditions provided", but in my specimens the abnormal one was no found in the specimens from the aquaria, which are believed to have bee in "bad condition."

In the *second* and the *third maxilliped* the distinction between the proximal segment of the endopodite and the distal of the protopodite is more o less obscure in later stages, and forms the basi-ischium article.

The succeeding five pair of segments appear after the second stage The following table shows the stages in which these appendages as well a the abdominal appendages come out.

As shown in the table of the next page the fifth pereiopod of *Leande paucidens* is uniramous through all the stages, and it comes out in a earlier stage than the preceding two pairs, while in *Paratya compressa* th thoracic appendages develop successively from the anterior pair to th posterior. The mode of the formation of the chela is similar in both o the species, and is different from that of *Palaemonetes varians* (5, 12, 20) i. e., the penultimate segment gradually produces inwards and forwards s as to form the immovable finger. In *Leander paucidens* the exopodite o the pereiopod degenerates as soon as it gives up its pelagic life, but a vestige of it remains until the ninth stage. On the other hand the exopodite is still noticeable on each leg of the adult in *Paratya compressa*. In this species it is a very interesting fact that the exopodite of each thoracic appendage as a rule bears two long setae at the distal extremity and two more on each margin, the longer one is closely in contact with the distal and the other is short and is a little behind the distal end at least in the early stages; though in the fifth pair of legs it attains the typical feature at the seventh stage; while in *Leander paucidens* it is entirely different in feature.

The *abdominal appendages*, at least the anterior five pairs, come out in a more or less later stage in *Paratya compressa* than those in *Leander paucidens*, and their developments in the former are also somewhat delayed compared to those of the latter.

As usual in Crustacea, the appendages of these shrimps typically consist of the protopodite, the exopodite, and the endopodite; but the appendages on each side of the mouth are much deformed especially in later

TABLE I.

Appendages / Larval stages	Pr 1	Pr 2	Pr 3	Pr 4	Pr 5	Pl 1–5	Pl 6
I	br br	br br	br r	r r	r r	— —	— —
II	bs bs	bs bs	bs br	br r	r ur	— —	— —
III	bs bs	bs bs	bs bs	bs br	br us	— —	br br
IV	bs bsc	bs bsc	bs bs	bs bs	bs us	— r	bs bs
V	bs bsc	bs bsc	bs bs	bs bs	bs us	r br	bs bs
VI	bsc bsc	bsc bsc	bs bs	bs bs	bs us	br bs	bs bs
VII	bsc bsc	bsc bsc	bs bs	bs bs	bs us	bs bs	bs bs
VIII	bsc bsc	bsc bsc	bs bs	bs bs	bs us	bs bs	bs bs
IX	bsc usc	bsc usc	bs us	bs us	bs us	bs bs	bs bs
X	bsc usc	bsc usc	bs us	bs us	bs us	bs bs	bs bs

Pr
1–5=The first to the fifth pereiopods, respectively.
Pl
1–5=The first to the fifth pleopods.
Pl
6=The sixth pleopod.

—=No trace of rudiment. r=Rudimentary. br=Not segmented, but biramous. bs=Biramous and segmented. ur=Uniramous and not segmented. us=Uniramous and segmented. bsc=Biramous, segmented, and chela provided. usc=Uniramous, segmented, and chela provided.

The upper line of each stage shows *Paratya compressa* and the lower line *Leander paucidens.*

stages or in the adult, and there are some conflicts of opinions in the use of the terms for various parts of the appendages (4, 6). Examining the features of the appendages through all the larval stages, we can see some characteristic changes in feature of the anterior appendages. For instance, the first maxilliped is similar in feature to the succeeding pairs in the

early stages, but with advancing development the exopodite is dilated and provided with some numbers of marginal setae; and it is more or less similar in feature to the outer foliaceous plate of the second maxilla. On the other hand in the second maxilla the anterior lobe of the inner four is provided with some numbers of setae on the inner margin as has been described above, and this is evidently the endopodite; and the remaining three or four lobes correspond to the protopodite. But in this appendage, does the posterior half of the outer foliaceous plate correspond or not to the epipodite? It is a difficult problem at present. In the first maxilla the anterior lobe is the endopodite and the exopodite is degenerated entirely in the present two species, though in some lower crustaceans a rudimentary one is noticeable (25, 30), and the proximal two lobes are the protopodite. If these observations are correct we can suppose that in the mandible the palp may be the endopodite, and the two processes, incisor and molar, the protopodite.

As has been described above, the setae on the exopodite of the thoracic appendages are characteristic in *Paratya compressa*. A similar feature of the exopodite has been described by E. VON DADAY (9) in the larval forms of *Caridina wyckii* (HICKSON) (*Caridina nilotica* ROUX); and this species as well as *Paratya compressa* belongs to the family Atyidae. This characteristic feature of the exopodite, I suppose, is probably common to the larvae of this family.

In both of the species the metamorphosis takes place very gradually, but in *Leander paucidens* some characteristic changes in feature occur between the seventh and the eighth larval stage. Prof. E. VON DADAY in his description of the metamorphosis of his species divided the larval stages into seven stages, i. e., 1, Euzoea, 2, Mesozoea, 3, Metazoea, 4, Protomysis, 5, Mesomysis, 6, Metamysis, 7, Postmysis; in one of the species, *Paratya compressa*, however, the changes of feature in each stage are so slight that the stages are not separable as are those of *Caridina wyckii*.

As a whole of the present two species *Paratya compressa* is primitive not only in the larva just hatched from the egg, but in the adult form as ORTMANN (24) rightly arranged the two families, Atyidae and Palaemonidae.

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EXPLANATION OF PLATES VIII—XV.

PLATE VIII.

Figs. 1–7. Posterior abdominal segment (telson) of Paratya compressa.

Fig. 1. First stage.

Fig. 2. Second stage.

Fig. 3. Third stage, showing 6th abdominal appendage. Branching cilia of setae omitted.

Fig. 4. Fourth stage, showing 6th abdominal appendage. Branching cilia of setae omitted.

Fig. 5. Fifth stage, showing 6th abdominal appendage. Branching cilia of setae omitted.

Fig. 6. Seventh stage, without 6th abdominal appendage. Branching cilia of setae omitted.

Fig. 7. Tenth stage, showing 6th abdominal appendage. Branching cilia of setae omitted.

Figs. 8–14. First antenna of *Paratya compressa.*

Fig. 8. First stage, dorsal view. Left side.

Fig. 9. Second stage, dorsal view. Left side.

Fig. 10. Third stage, ventral view. Left side.

Fig. 11. Fourth stage, ventral view. Right side.

Fig. 12. Sixth stage, ventral view. Right side.

Fig. 13. Seventh stage, dorsal view. Left side.

Fig. 14. Tenth stage, dorsal view. Left side.

Figs. 15–21. Second antenna of *Paratya compressa.*

Fig. 15. First stage, ventral view. Right side. The distal part of the endopodite is much enlarged.

Fig. 16. Second stage, ventral view. Left side. The distal part of the endopodite is much enlarged.

Fig. 17. Third stage, dorsal view. Right side.

Fig. 18. Fourth stage, ventral view. Right side.

Fig. 19. Sixth stage, ventral view. Right side.

Fig. 20. Seventh stage, ventral view. Right side.

Fig. 21. Tenth stage, dorsal view. Left side.

Figs. 22–24. Mandible of *Paratya compressa.*

Fig. 22. First stage. Left side.

Fig. 23. Second stage. Right side.

Fig. 24. Third stage. Right side.

Figs. 25–29. First maxilla of *Paratya compressa.* (Figs. 25 & 26 are more enlarged than the others).

Fig. 25. First stage. Left side.

Fig. 26. Second stage. Right side.

Fig. 27. Eighth stage. Right side.

Fig. 28. Ninth stage. Right side.

Fig 29. Tenth stage. Right side.

PLATE IX.

Fig. 30–37. Second maxilla of *Paratya compressa.*

Fig. 30. First stage.

Fig. 31. Second stage.

Fig. 32. Third stage.

Fig. 33. Fourth stage.

Fig. 34. Fifth stage.

Fig. 35. Eighth stage.

Fig. 36. Ninth stage.

Fig. 37. Tenth stage.

Figs. 38–46. First maxilliped of *Paratya compressa.*

Fig. 38. First stage.

Fig. 39. Second stage.

Fig. 40. Third stage.

Fig. 41. Fourth stage.

Fig. 42. Fifth stage.

Fig. 43. Sixth stage.

Fig. 44. Eighth stage.

Fig. 45. Ninth stage.

Fig. 46. Tenth stage. The endopodite is partly much enlarged.

Figs. 47–51. Second maxilliped of *Paratya compressa.*

Fig. 47. First stage.

Fig. 48. Second stage.

Fig. 49. Eighth stage.

Fig. 50. Ninth stage.

Fig. 51. Tenth stage.

Fig. 52-57. Third maxilliped of *Paratya compressa*.
Fig. 52. First stage.
Eig. 53. Second stage.
Fig. 54. Fifth stage.
Fig. 55. Eighth stage.
Fig. 56. Ninth stage.
Fig. 57. Tenth stage.
Figs. 58-64. First pereiopod of *Paratya compressa*.
Fig. 58. First stage.
Fig. 59. Second stage.
Fig. 60. Fifth stage.
Fig. 61. Sixth stage.
Fig. 62. Eighth stage.
Fig. 63. Ninth stage.
Fig. 64. Tenth stage, viewed from out side. Left side.
Figs. 65-69. Second pereiopod of *Paratya compressa*.
Fig. 65. Second stage.
Fig. 66. Fifth stage.
Fig. 67. Eighth stage.
Fig. 68. Ninth stage.
Fig. 69. Tenth stage.
Figs. 70-74. Third pereioped of *Paratya compressa*.
Fig. 70. Second stage.
Fig. 71. Sixth stage.
Fig. 72. Seventh stage.
Fig. 73. Eighth stage.
Fig. 74. Ninth stage.

PLATE X.

Figs. 75-81. Fourth pereiopod of *Paratya compressa*.

Fig. 75. Third stage (a little more enlarged than the others). The terminal part of .he exopodite is more enlarged.

Fig. 76. Fourth stage.
Fig. 77. Fifth stage.
Fig. 78. Sixth stage.
Fig. 79. Eighth stage.
Fig. 80. Ninth stage.
Fig. 81. Tenth stage.
Figs. 82-87. Fifth pereiopod of *Paratya compressa*.
Fig. 82. Third stage (more magnified).
Fig. 83. Fourth stage.
Fig. 84. Seventh stage.
Fig. 85. Eighth stage.
Fig. 86. Ninth stage.
Fig. 87. Tenth stage.
Figs. 88-92. First pleopod of *Paratya compressa*.
Fig. 88. Sixth stage, more enlarged than those of the succeeding stages.

Fig. 89. Seventh stage.
Fig. 90. Eighth stage.
Fig. 91. Ninth stage.
Fig. 92. Tenth stage, a little less enlarged.
Figs. 93–97. Second pleopod of *Paratya compressa*.
Fig. 93. Sixth stage, more enlarged than those of the succeeding stages.
Fig. 94. Seventh stage.
Fig. 95. Eighth stage.
Fig. 96. Ninth stage.
Fig. 97. Tenth stage, a little less enlarged.
Figs. 98–107. Telson of *Leander paucidens*.
Fig. 98. First stage.
Fig. 99. Second stage, branching cilia of the setae omitted.
Fig. 100. Third stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 101. Fourth stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 102. Fifth stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 103. Sixth stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 104. Seventh stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 105. Eighth stage, showing sixth abdominal appendage. Branching cilia of the setae omitted.
Fig. 106. Ninth stage, showing terminal part of telson.
Fig. 107. Tenth stage, showing terminal part of telson.

PLATE XI.

Figs. 108–117. First antenna of *Leander paucidens*.
Fig. 108. First stage, dorsal view. Left side.
Fig. 109. Second stage, ventral view. Left side.
Fig. 110. Third stage, dorsal view. Right side.
Fig. 111. Fourth stage, somewhat obliquely ventral view. Left side
Fig. 112. Fifth stage, dorsal view. Left side.
Fig. 113. Sixth stage, dorsal view. Left side.
Fig. 114. Seventh stage, dorsal view. Left side.
Eig. 115. Eighth stage, dorsal view. Left side.
Fig. 116. Ninth stage, dorsal view. Left side, less enlarged.
Fig. 117. Tenth stage, dorsal view. Left side, less enlarged.
Figs. 118–124. Second antenna of *Leander paucidens*.
Fig. 118. First stage, dorsal view. Right side.
Fig. 119. Second stage, dorsal view. Left side.
Fig. 120. Third stage, dorsal view. Left side.
Fig. 121. Fourth stage, dorsal view. Left side.
Fig. 122. Fifth stage, ventral view. Right side.
Fig. 123. Sixth stage, ventral view. Right side.

Fig. 124. Eighth stage, ventral view. Left side. The terminal part of flagellum is discarded.

Figs. 125–134. Inner margin of mandible of *Leander paucidens.*

Fig. 125. First stage. Viewed from inner side, right side.

Fig. 126. Second stage, inner view. Left side.

Fig. 127. Third stage, outer view. Left side.

Fig. 128. Fourth stage, outer view. Right side.

Fig. 129. Fifth stage, outer view. Right side.

Fig. 130. Sixth stage, outer view. Right side.

Fig. 131. Seventh stage, outer view. Left side.

Fig. 132. Eighth stage, inner view. Left side.

Fig. 133. Ninth stage, inner view. Left side.

Fig. 134. Tenth stage, inner view. Left side.

Plate XII.

Figs. 135–143. First maxilla of *Leander paucidens.*

Fig. 135. First stage.

Fig. 136. Second stage.

Fig. 137. Third stage.

Fig. 138. Fourth stage, less enlarged.

Fig. 139. Sixth stage.

Fig. 140. Seventh stage.

Fig. 141. Eighth stage, less enlarged.

Fig. 142. Ninth stage, less enlarged.

Fig. 143. Tenth stage, less enlarged.

Figs. 144–152. Second maxilla of *Leander paucidens.*

Fig. 144. First stage.

Fig. 145. Second stage.

Fig. 146. Fourth stage.

Fig. 147. Fifth stage.

Fig. 148. Sixth stage.

Fig. 149. Seventh stage.

Fig. 150. Eighth stage.

Fig. 151. Ninth stage.

Fig. 152. Tenth stage.

Figs. 153–162. First maxilliped of *Leander paucidens.*

Fig. 153. First stage.

Fig. 154. Second stage.

Fig. 155. Third stage.

Fig. 156. Fourth stage.

Fig. 157. Fifth stage.

Fig. 158. Sixth stage.

Fig. 159. Seventh stage.

Fig 160. Eighth stage.

Fig. 161. Ninth stage.

Fig. 162. Tenth stage.

PLATE XIII.

Figs. 162–172. Second maxilliped of *Leander paucidens.*
Fig. 163. First stage.
Fig. 164. Second stage.
Fig. 165. Third stage.
Fig. 166. Fourth stage.
Fig. 167. Fifth stage.
Fig. 168. Sixth stage.
Fig. 169. Seventh stage.
Fig. 170. Eighth stage.
Fig. 171. Ninth stage.
Fig. 172. Tenth stage.
Figs. 173–183. Third maxilliped of *Leander paucidens*
Fig. 173. First stage.
Fig. 174. Second stage.
Fig. 175. Third stage.
Fig. 176. Fourth stage.
Fig. 177. Fifth stage.
Fig. 178. Sixth stage.
Fig. 179. Seventh stage.
Fig. 180. Eighth stage.
Fig. 181. Ninth stage.
Fig. 182. Tenth stage.
Figs. 183–191. First pereiopod of *Leander paucidens.*
Fig. 183. Second stage.
Fig. 184. Third stage.
Fig. 185. Fourth stage.
Fig. 186. Fifth stage.
Fig. 187. Sixth stage.
Fig. 188. Seventh stage.
Fig. 189. Eighth stage.
Fig. 190. Ninth stage less enlarged.
Fig. 191. Tenth stage less enlarged.

PLATE XIV.

Figs. 192–200. Second pereiopod of *Leander paucidens.*
Fig. 192. Second stage.
Fig. 193. Third stage.
Fig. 194. Fourth stage.
Fig. 195. Fifth stage.
Fig. 196. Sixth stage.
Fig. 197. Seventh stage.
Fig. 198. Eighth stage.
Fig. 199. Ninth stage, less enlarged.
Fig. 200. Tenth stage, less enlarged.
Figs. 201–209. Third pereiopod of *Leander paucidens.*

Fig. 201. Second stage.
Fig. 202. Third stage.
Fig. 203. Fourth stage.
Fig. 204. Fifth stage.
Fig. 205. Sixth stage.
Fig. 206. Seventh stage.
Fig. 207. Eighth stage.
Fig. 208. Ninth stage, less enlarged.
Fig. 209. Tenth stage, less enlarged.
Figs. 210–215. Fourth pereiopod of *Leander paucidens*.
Fig. 210. Fourth stage.
Fig. 211. Fifth stage.
Fig. 212. Sixth stage.
Fig. 213. Seventh stage.
Fig. 214. Eighth stage.
Fig. 215. Ninth stage, less enlarged.
Figs. 216–223. Fifth pereiopod of *Leander paucidens*.
Fig. 216. Second stage, a little more enlarged. Dotted line shows the contour of development in the next stage.
Fig. 217. Third stage.
Fig. 218. Fourth stage.
Fig. 219. Fifth stage.
Fig. 220. Sixth stage, less enlarged.
Fig. 221. Seventh stage, less enlarged.
Fig. 222. Eighth stage, less enlarged.
Fig. 223. Ninth stage, less enlarged.

Plate XV.

Figs. 224–230. Pleopods of *Leander paucidens*.
Fig. 224. Fourth stage, more enlarged than the others.
Figs. 225–230. First pair of pleopods.
Fig. 225. Fifth stage.
Fig. 226. Sixth stage.
Fig. 227. Seventh stage.
Fig. 228. Eighth stage.
Fig. 229. Ninth stage, less enlarged.
Fig. 230. Tenth stage, more enlarged than Fig. 229, but not as much as Figs. 225–228.
Figs. 231–236. Second pair of pleopods.
Fig. 231. Fifth stage.
Fig. 232. Sixth stage.
Fig. 233. Seventh stage.
Fig. 234. Eighth stage.
Fig. 235. Ninth stage, as much as Fig. 229 enlarged.
Fig. 236. Tenth stage, as much as Fig. 230 enlarged.

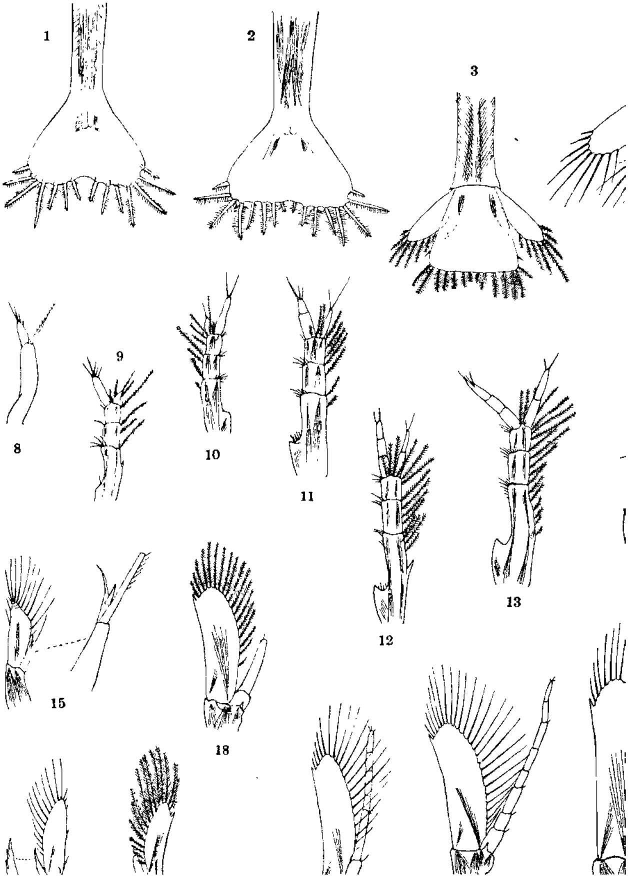
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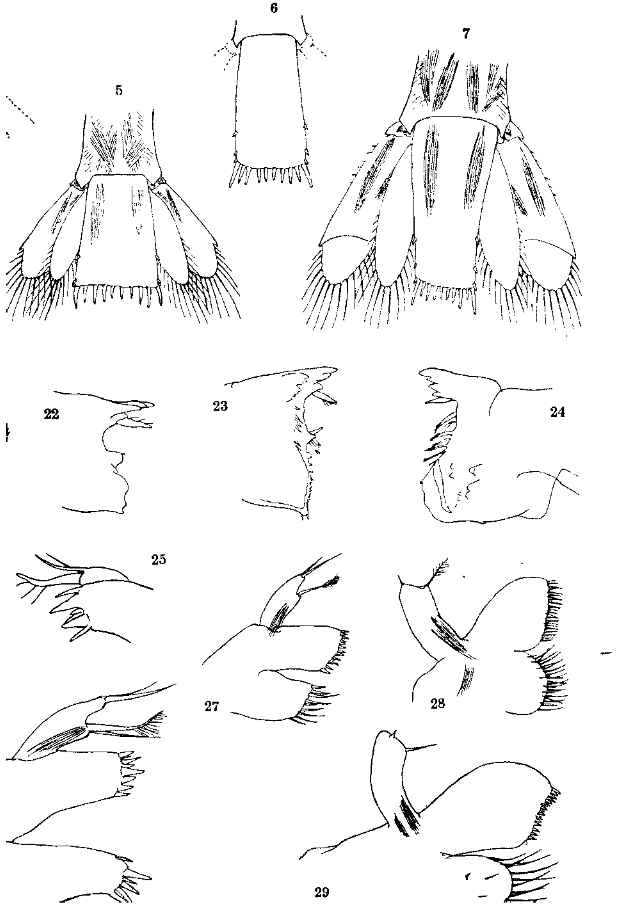
Plate VIII.



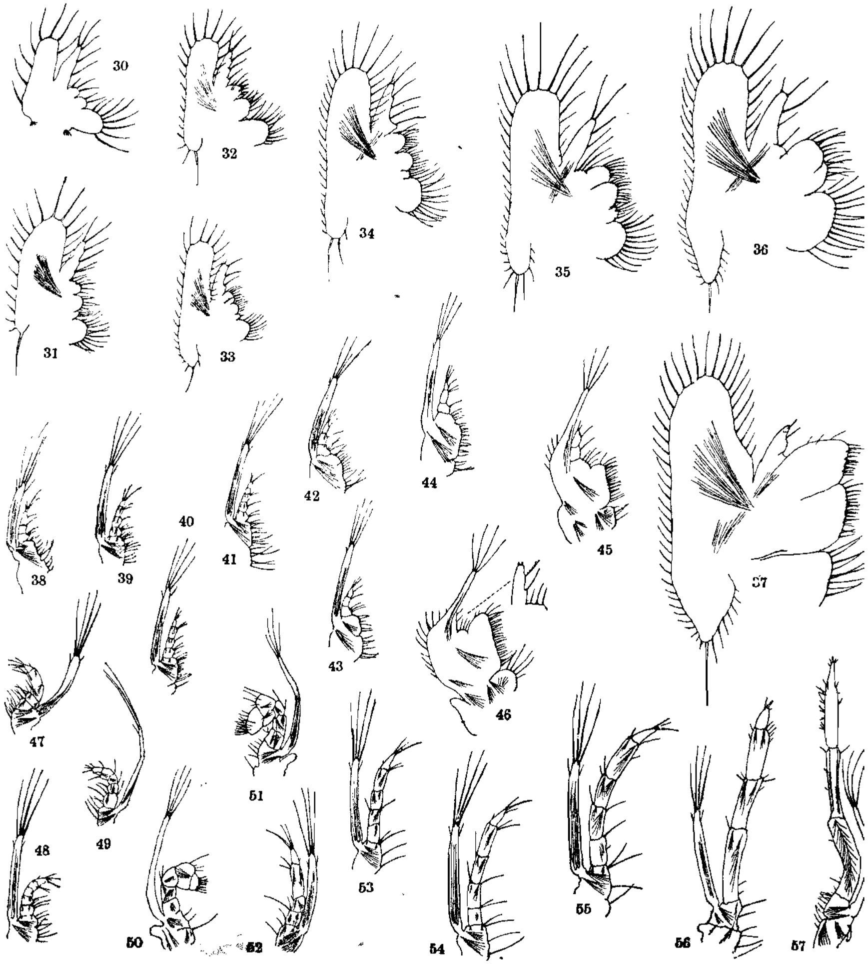

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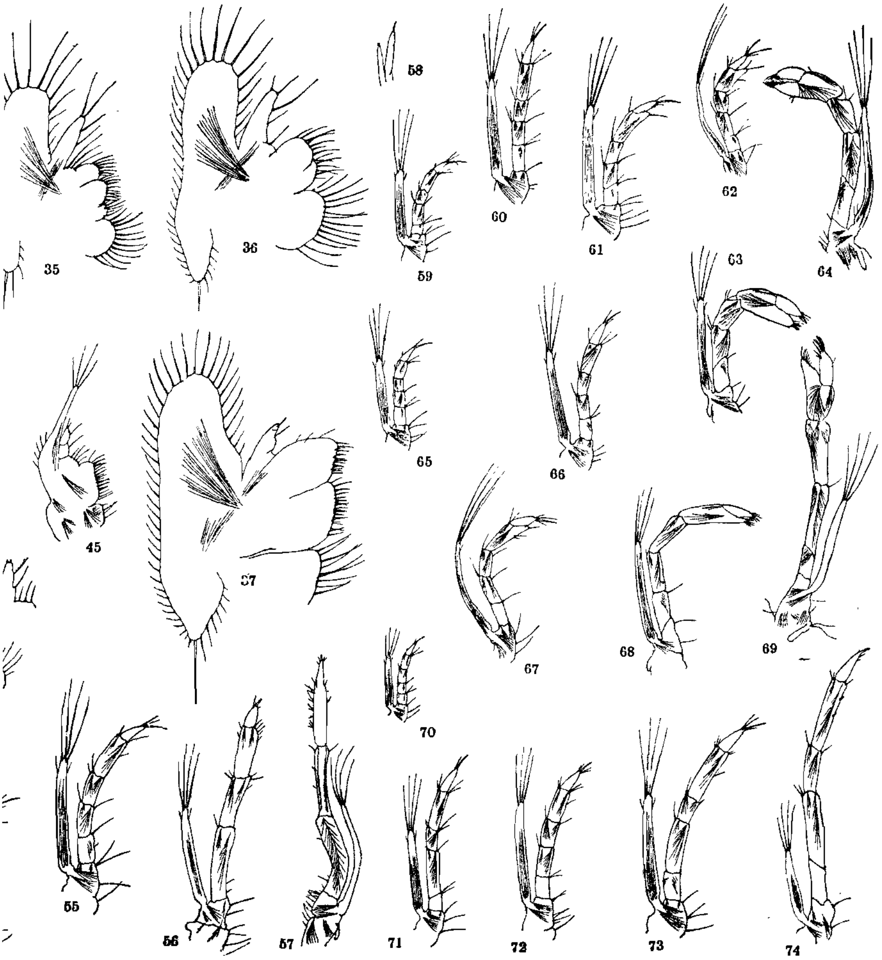
Plate IX.



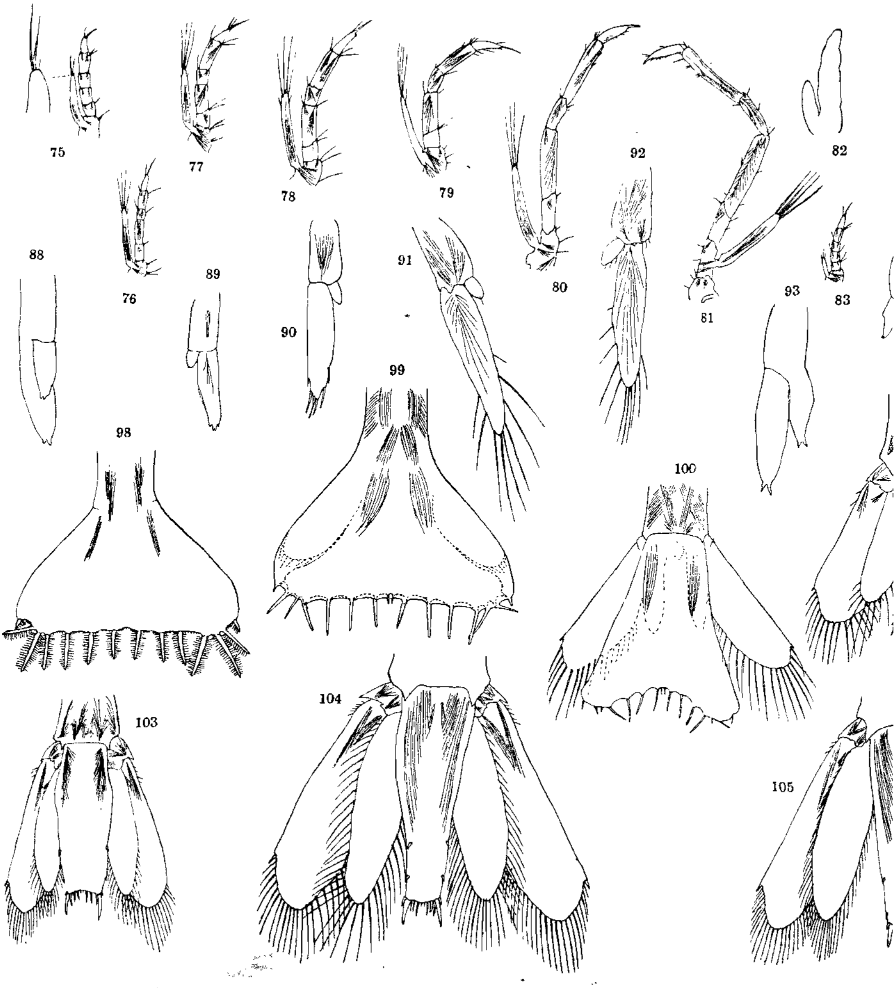


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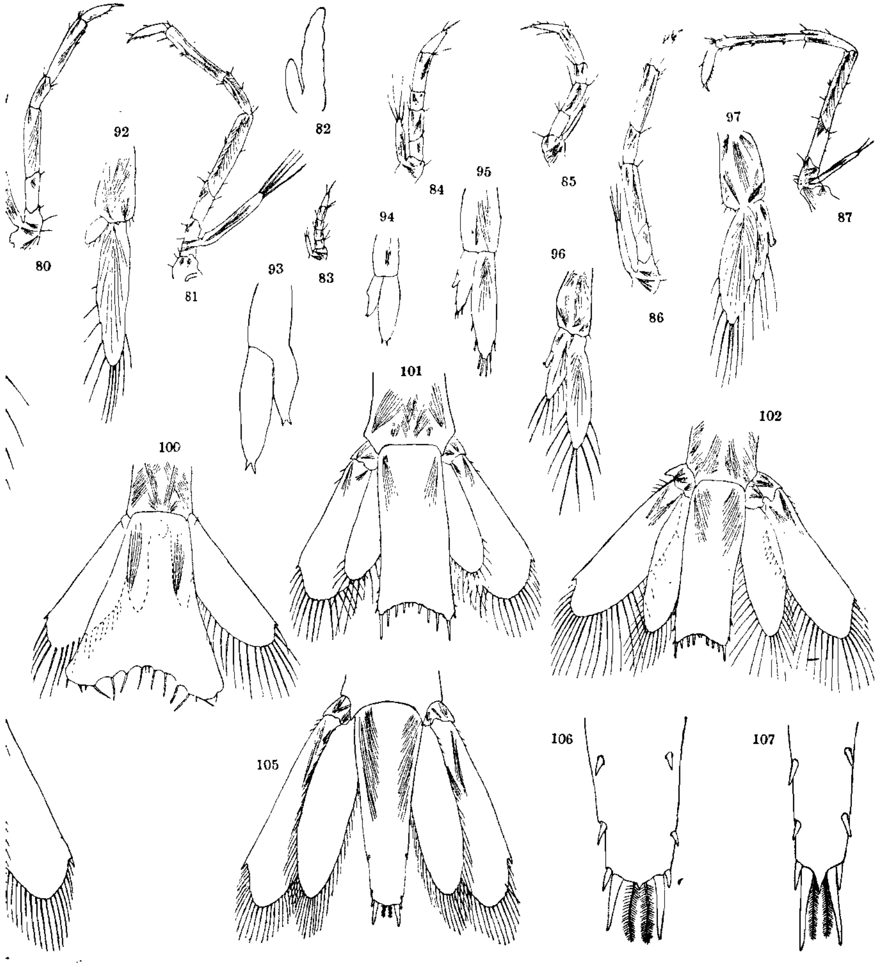
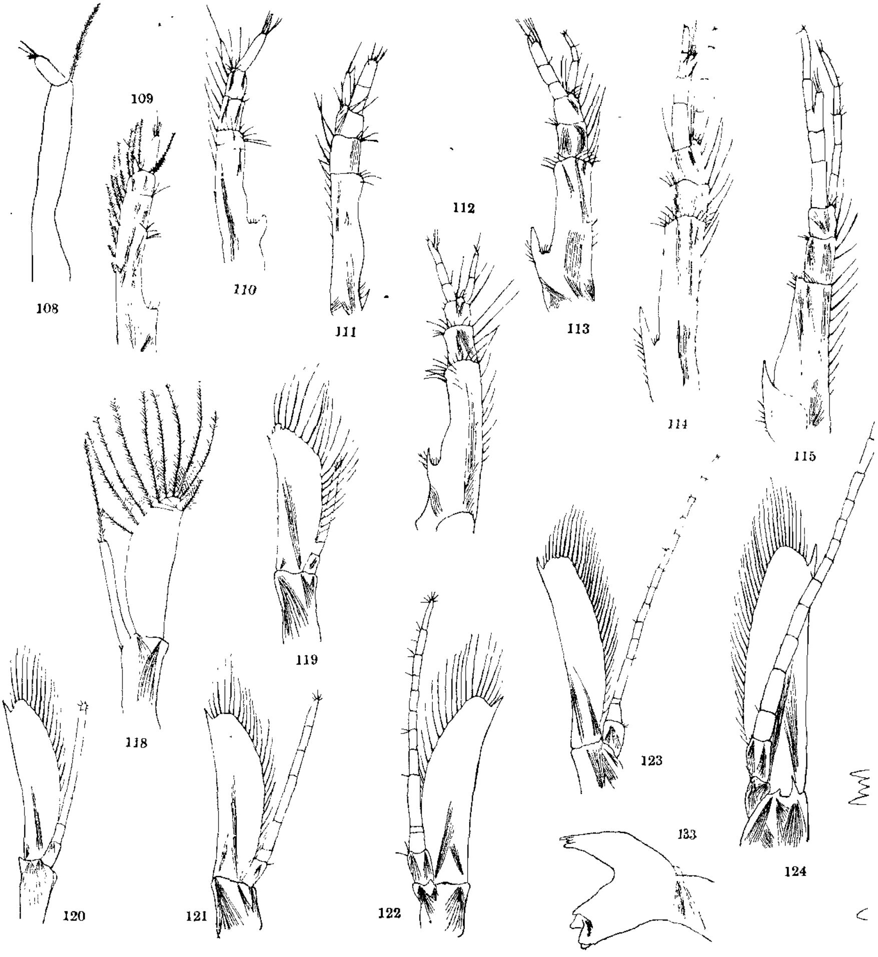
Plate X.

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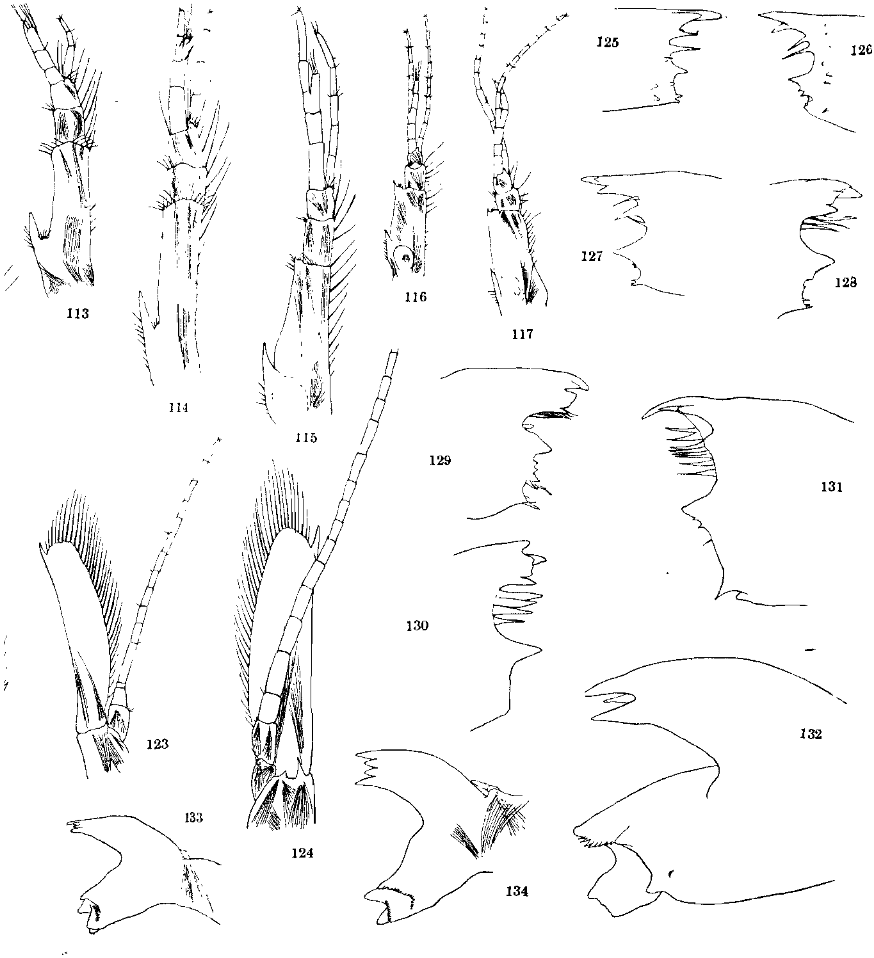
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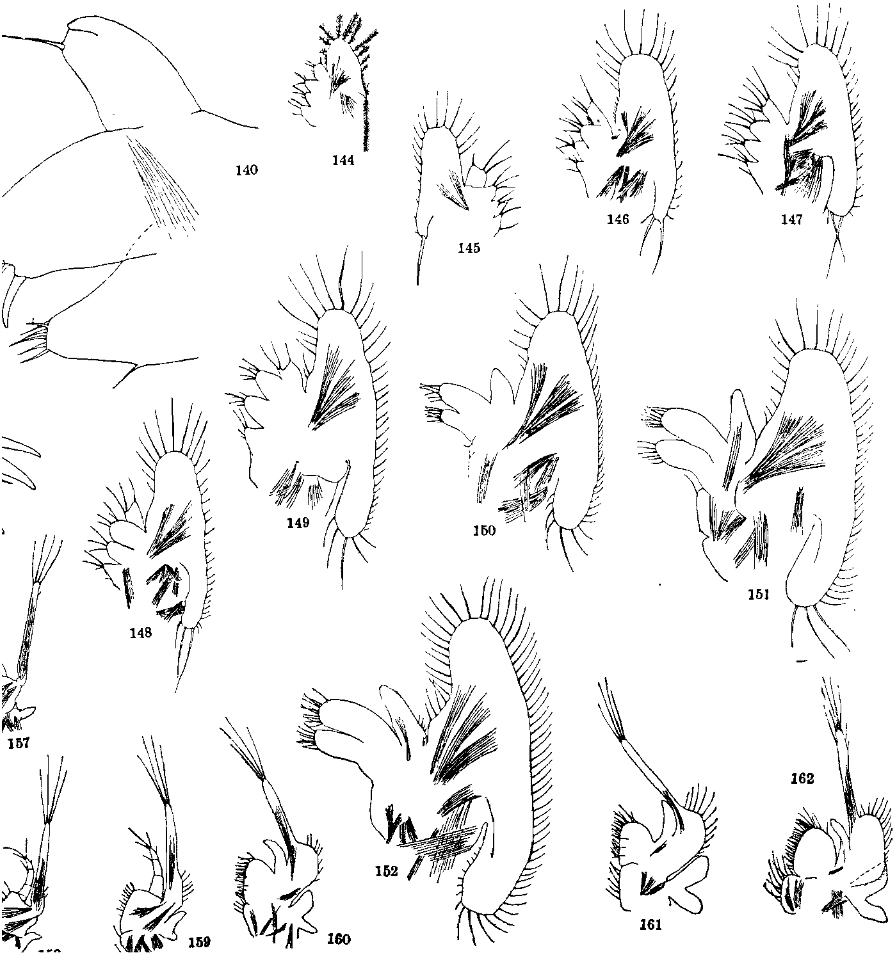

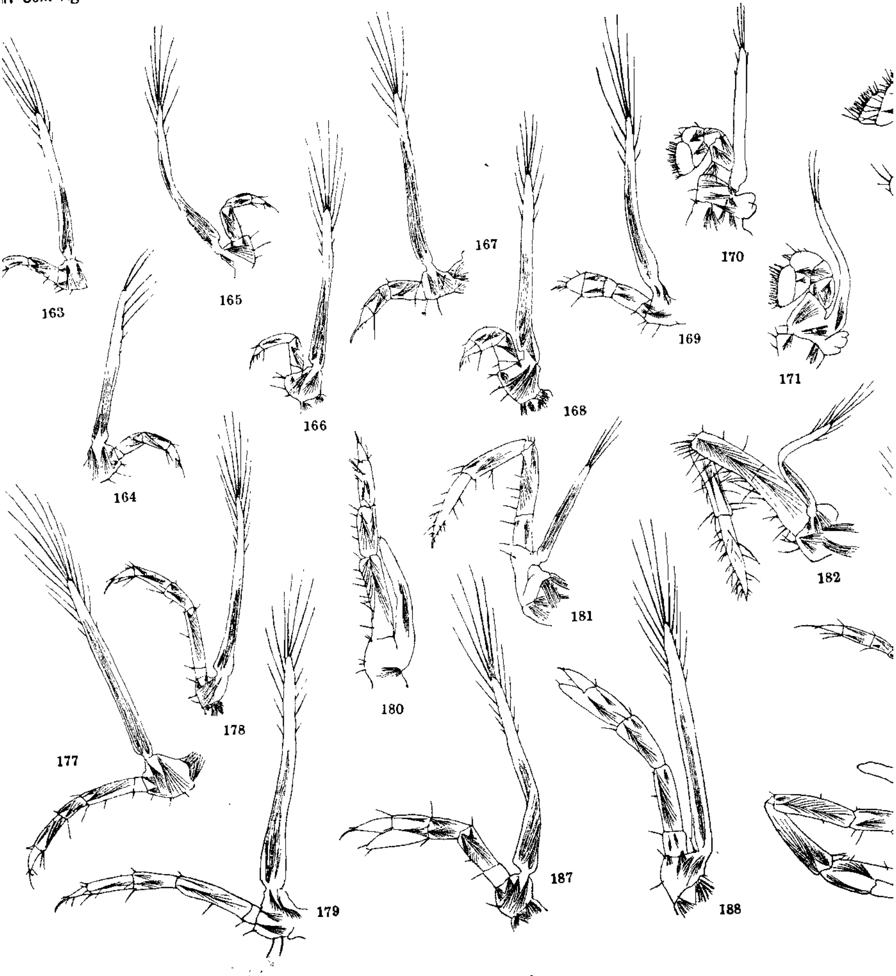
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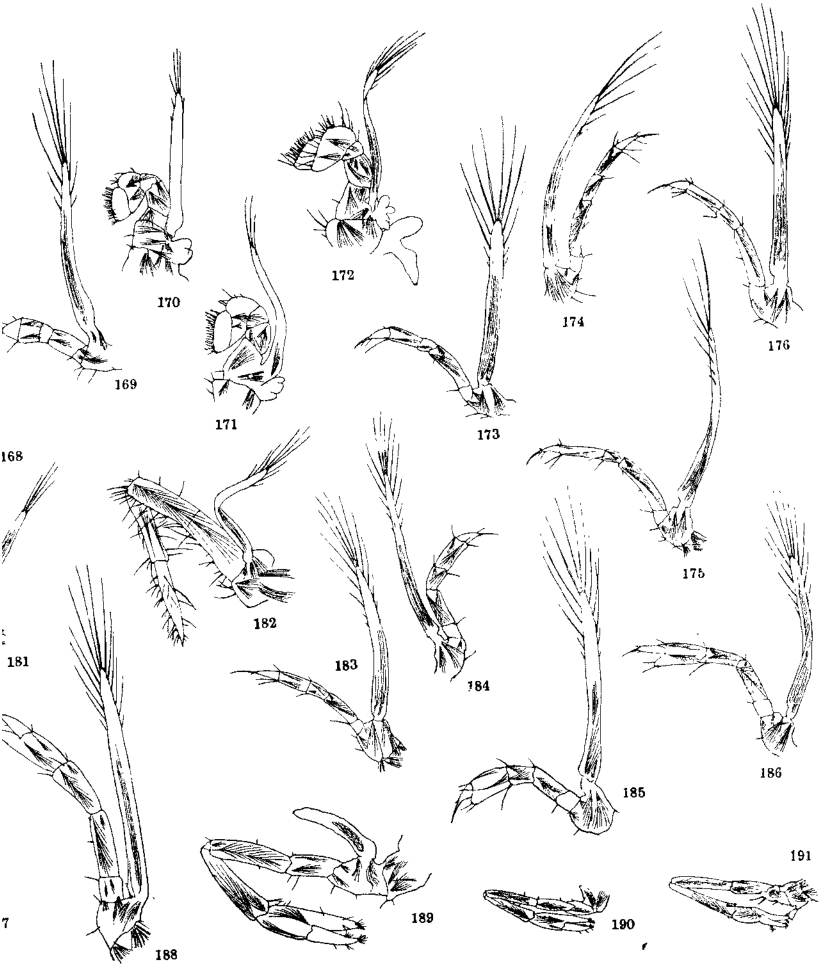
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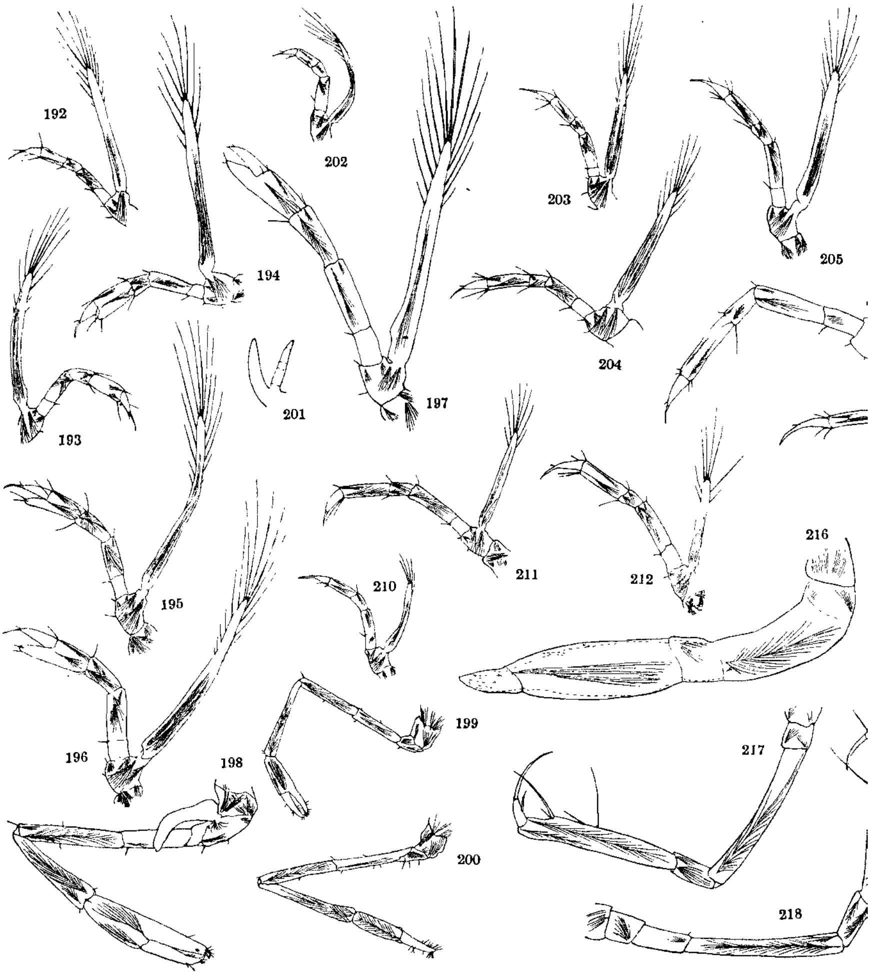
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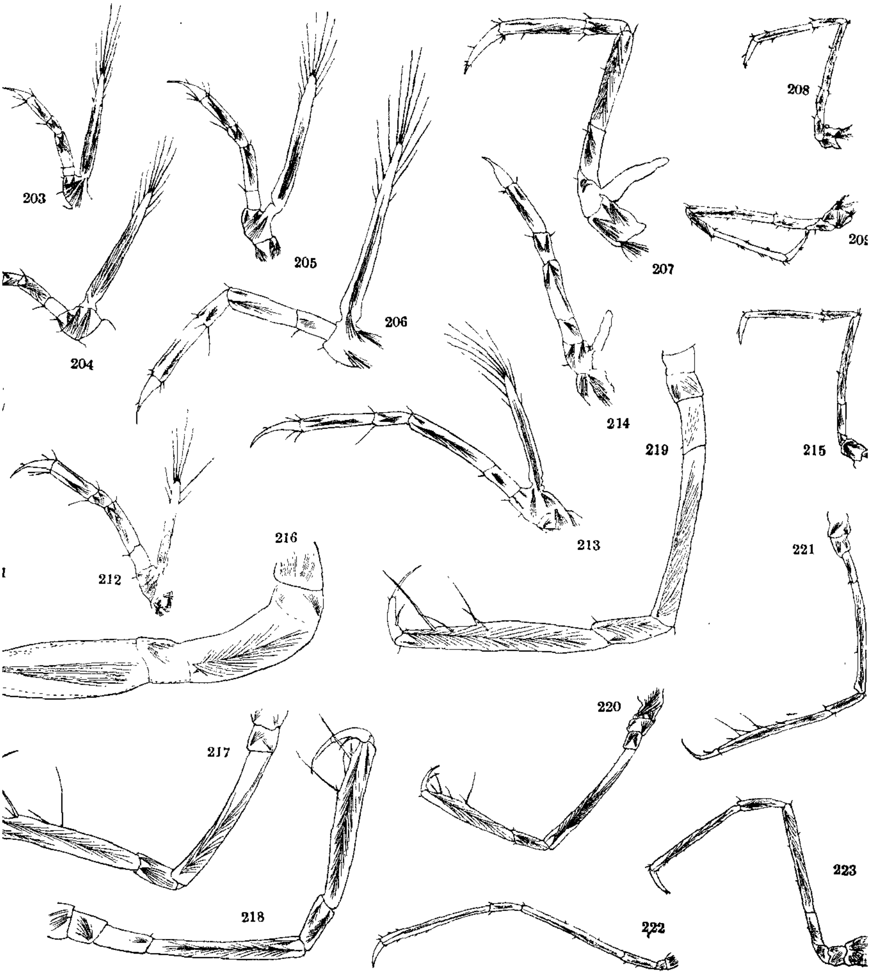
Plate XIV.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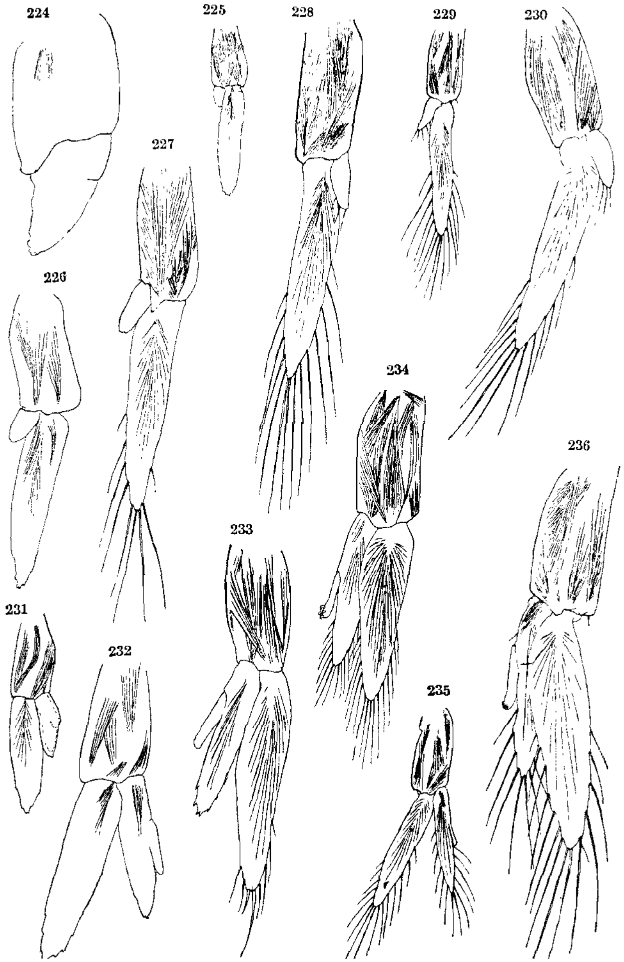
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Untersuchungen über die Larve der Kiefernblattwespe, Nesodipron japonica Marlatt.

Von

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der Land- und Forstwirtschaftlichen Hochschule zu Miyazaki.

Mit 78 Abbildungen und 2 Tabellen im Text.

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Einleitung.

Das enorme Auftreten der Blattwespenlarven (*Nesodipron japonica* MARLATT) auf den jungen Pfläuzchen von *Pinus densiflora* SIEB. et ZUCC. (Aka-matu) in der Pflanzschule der hiesigen Hochschule hat mich im vorigen Jahre veranlasst, eine vergleichende Studie derselben in Bezug auf die Seidenraupe vorzunehmen. Alle Kiefernpflänzchen wurden dabei fast kahl gefressen, und selbst die verschonten Blätter wurden dürr und fielen ab, sodass manche Sprösslinge schliesslich sogar zugrunde gingen, ohne sich von den Schäden erholen und im nächsten Frühjahr neue Sprossen austreiben zu können Da es aber im Larvenzustande ist, dass die Wespen Pflanzen schädigen, so habe ich mich im folgenden hauptsächlich nur auf dieses Stadium beschränkt, und zwar besonders die makro- und mikroskopischen Strukturen äusserer und innerer Organe etwas eingehend untersucht, zumal da in ihnen, im Vergleich mit der Seidenraupe, mancherlei interessante Übereinstimmungen zu erblicken sind. In der Tat zeigt die Blattwespenlarve in ihrem Körperbau zahlreiche merkwürdige Anklänge an die Seidenraupe, welche als von gleichen ökologischen Bedingungen hervorgerufene sekundäre Anpassungen gedeutet werden könnten. Es handelt sich also hier um einen Fall der sogenannten Konvergenzerscheinungen. Indessen sind doch der furchtbaren Schädlichkeit wegen auch einige pflanzenschutzliche Massregeln am Schluss der biologischen Bemerkungen erwähnt worden, die bei der Schädlingsbekämpfung wirksam wären.

Es ist mir eine angenehme Pflicht, meinem hochgeschätzten Lehrer, Herrn Prof. TOKIO KABURAKI, unter dessen beständiger Leitung ich diese Arbeit ausgeführt habe, meinen herzlichsten Dank auszusprechen.

Äussere Morphologie.

I. KÖRPERÜBERSICHT.

Zu allerlei morphologischen Untersuchungen wählte ich die ausgewachsenen, präpupalen Larven. Der Körper setzt sich aus einer, mit einer

kräftigen, der Kaumuskulatur als Widerhalt dienenden und das Nervenzentrum (das Gehirn) schützend einschliessenden Chitinpanzerung versehenen, rot-bräunlich gefärbten Kopfkapsel und einer sich daran schliessenden Reihe von dreizehen weichen, dunkelgrünen, auf der Rückenseite aber dazu noch etwas gebräunten Hinterleibsringen zusammen. Die ersten drei Segmente bilden den Thorax, die übrigen das Abdomen, am Ende des letzteren, am After mündet der mit dem Munde auf der Unterseite des Kopfes beginnende, durch den ganzen Körper hindurch geradläufig ziehende Speisekanal aus. Die einzelnen Hinterleibsringe zeigen wiederum mehrere, noch feinere Ringelungen von oberflächlicher Natur, welche aber auf den ersten Blick mit einem wahren Segment verwechselt werden könnten, oder durch deren Vorhandensein wenigstens die echten Segmentgrenzen beträchtlich obskuriert werden.

Die Länge der vollgereiften Larve beträgt ca 2 cm. Der Körper erscheint im allgemeinen dunkelgrün, sodass er sich zwischen den Blattnadeln nur mit Schwierigkeit von der Umgebung unterscheiden lässt. Am Kopf sowie auch um den After ist er aber rötlichbraun gefärbt. Zu beiden Seiten des Kopfes ist je ein grosser Genalfleck vorhanden, in dessen Mitte ein wohl entwickeltes, dunkles, halbkugeliges Punktauge zu bemerken ist. Die sechs kohlschwarzen Brustbeine bleiben an den Gelenkstellen zwischen den Gliedern frei von Pigment. Die Abdominalfüsse, von denen acht Paare vorhanden sind, sind demgegenüber, wie die Bauchfläche des Körpers selbst, leicht gelblich grün anzusehen. Die tiefbraunen, kräftig entwickelten Mandibeln der Mundwerkzeuge sind zum Abnagen der Nährblätter an den inneren Rändern mit je fünf starken Zähnen ausgerüstet.

Wie schon erwähnt, haben die drei Thoraxsegmente je ein Paar Brustbeine; auch den Abdominalringen kommen, mit Ausnahme des ersten und des neunten, je ein Paar fleischige, sackförmige Ausstülpungen, die Abdominalfüsse, zu, sodass sich am ganzen Hinterleibe insgesamt zweiundzwanzig Füsse abzählen lassen. Im Gegensatz zu den Abdominalfüssen, sind die Brustbeine deutlich gegliedert. Sie gestalten sich bei der Verpuppung zu den Beinen des Imagos um, während die ersteren dabei völlig verschwinden. An jedem Segment kommen ferner ein Paar winzige, fast mikroskopische Stigmen auf beiden Seiten vor. Nur fehlen sie vollständig an dem Meso- und Metathorax sowie auch an den letzten zwei Segmenten des Abdomens. Im ganzen genommen bietet also die gesamte Körperkonstruktion der Blattwespenlarve, mit der der Seidenraupe verglichen, eine schlagende Ähnlichkeit, nur abgesehen davon, dass die Zahl und Verteilung der Abdominalfüsse zwischen beiden Arten ziemlich verschieden ist, dass die Blattwespenlarve

im Gegensatz zu der Seidenraupe keinen Dorsalfortsatz in der kaudalen Gegend hat, und dass in der letzteren das neunte Abdominalsegment niemals so atrophiert wie bei der ersteren, sondern vollkommen ausgebildet ist.

Tabelle I. Die Übersicht der Rumpfgliederung.
I-III......Segmente des Thoraxes,
1-10 ......dieselben des Abdomens,
B ....Brustbein,
F ....Abdominalfuss,
s............Stigma.

I	II	III	1	2	3	4	5	6	7	8	9	10
s			s	s	s	s	s	s	s	s		
B	B	B		F	F	F	F	F	F	F		F

II. Der Kopf.

Die Chitinkapsel des Kopfes ist, wie die der Seidenraupe, in drei Regionen geteilt, von denen die beiden Scheitelplatten den grösseren Teil des Kopfes ausmachen. Die Medianfurche zwischen den beiden Platten gabelt sich nach unten in Form eines umgekehrten „Y“ s, sodass von ihr auf der Stirn das Stirndreieck ausgeschnitten wird, zwischen welchem und der Oberlippe der Mundwerkzeuge der schmale Kopfschild eingeschaltet ist. Auf der Unterseite des Kopfes finden sich zu beiden Seiten die wichtigsten Sinnesorgane, namentlich ein Fühler und oberhalb desselben ein starkes Punktauge (Abb. 1). Sie liegen, wie es auch bei der Seidenraupe der Fall ist, auf der Scheitelplatte, und zwar dicht an die untere Ecke angerückt. Anstatt der sechs Ocellen der genannten Raupe ist hier aber das Sehorgan von einem einzigen, gewaltig entwickelten Punktauge vertreten, ringsum welches die Oberfläche der Scheitelplatte in einem ziemlich weiten Umfang tiefschwarz gefleckt ist. Dieses erinnert mich sehr an den Genalfleck der Seidenraupen von der Sorte „Zebra“ (Tora-ko).

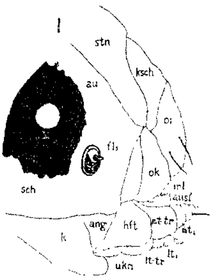


Abb. 1. Die Mundteile, von der rechten Seite gesehen.
ang Angelglied, au Auge, ausl Aussenlade der Maxille, fl_3 Endglied des Fühlers, *hft* Haftglied, inl Innenlade der Maxille, k Kehle, ksch Kopfschild, lt_1 1. Glied des Tasters der Unterlippe, lt-tr Tasterträger derselben, mt_1 1. Glied des Tasters des Maxille, mt-tr Tasterträger derselben, *ok* Oberkiefer, *ol* Oberlippe, sch Scheitelplatte, stn Stirnplatte, ukn Unterkinn.

Trotz gewisser Ähnlichkeiten im Aussehen ist aber der Fühler der Wespenlarve, mit dem der Seidenraupe verglichen, ganz abweichend gestaltet. Er besteht, nach meiner Meinung, aus drei Gliedern, von denen sich das Endglied in Gestalt eines kegelförmigen Zapfens inmitten eines elliptischen, zarthäutigen Feldes erhebt, welch letzteres aus den zwei anderen, stark abgeplatteten Gliedern zusammengesetzt zu sein scheint. Die beiden Glieder zusammen umgeben einerseits die Basis des konischen Endgliedes konzentrisch, andererseits nehmen sie auf der Scheitelplatte ein scharf abgegrenztes, elliptisches Feldstückchen ein. Auf denselben Gliedern liegen ferner im unteren, stark pigmentierten Teile je zwei kraterförmige Einsenkungen, welche als mächtig ausgebildete Geruchslöcher zu deuten sind. Das Endglied ist aus festem, schwärzlichem Chitin gebildet. Die ganze Oberfläche des Kopfes ist, wie die der Seidenraupe, ziemlich dicht behaart.

Auch in der relativen Lage und in der Beschaffenheit der einzelnen Mundwerkzeuge sind auffällige Ähnlichkeiten zwischen den Seidenraupen und den Wespenlarven zu erblicken (Abb. 2). So ist hier die Oberlippe ebenfalls eine halbkreisförmige Platte mit einem medianen Ausschnitt, auf deren Oberseite vier ziemlich lange, in gleicher Entfernung voneinander auf einer Querlinie angereihte Haare vorkommen. Die Mandibeln, die *sich unter der Oberlippe befinden, sind* sogar noch gewaltiger ausgebildet als die der Seidenraupe, was sich nur leicht verstehen lässt, wenn man an die harte, xelophytische Beschaffenheit der zu bearbeitenden Nadelblätter denkt.

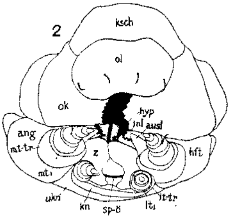


Abb. 2. Die Mundteile, von unten gesehen.
hyp Hypopharynx, kn Kinn, sp-ö Spinndrüsenöffnung, z Zunge, die übrigen Bezeichnungen wie in Abb. 1.

An den Maxillen (Abb. 3. u. 4) sind ebenfalls die zwei gut ausgebildeten, häutigen Glieder zu unterscheiden, d. i., das Angelglied (Cardo) und das Haftglied (Stipes), von denen das letztere noch zwei distale Fortsätze trägt, und zwar einerseits (median) die Kaulade, andererseits (lateral) den Taster (Palpus maxillaris), der vermittelst des Tasterträgers (Palpifer) an das Haftglied geknüpft ist. Anstatt der einheitlichen, sackförmigen Anschwellung der Seidenraupe ist hier die Kaulade in die Innen- und Aussenladen gespaltet, von denen die erstere mit einem grösseren und sechs kleineren, zusammen in einer Reihe geordneten Sinneskegeln ausgerüstet ist. Was den Taster betrifft, so ist er hier aus vier sich distal rasch verjüng-

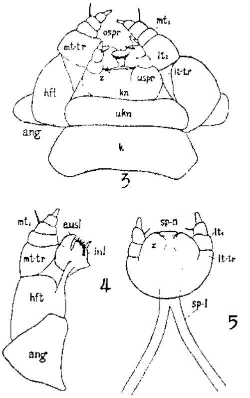


Abb. 3. Die Mundteile, von hinten gesehen.
Abb. 4. Die linke Maxille, von vorn gesehen.
Abb. 5. Die Unterlippe, von vorn gesehen.
ospr obere (vordere) Wand der Spinnröhre (Spinnwarze), sp-l Spinndrüsenleiter, uspr untere (hintere) Wand der Spinnröhre, weitere Bezeichnungen wie in Abb. 1–2.

enden Artikeln zusammengesetzt, während bei dem der Seidenraupe nur drei Glieder zu bemerken sind. Der Tasterträger der Blattwespenlarve ist mit einem kleinen Haare besetzt. Das einzige Haar des Tasters kommt dem zweiten Artikel zu.

Wie es bei den meisten Insekten der Fall ist, so ist auch hier die Unterlippe (Abb. 3 u 5) durch Verwachsen der beiden zweiten Maxillen zustande gekommen, sodass ihr Bau im wesentlichen mit dem der ersten Maxille übereinstimmt. Die Sachlage ist bei der Seidenraupe auch dieselbe; nur ist dort das Zusammenwachsen insofern noch weiter vorgeschritten, als zwischen den beiden Hälften keine Suturfurche mehr hinterlassen ist. In der Wespenlarve ist diese Furche auf der Innenseite der Unterlippe deutlich aufgewiesen. Wo sie mit dem distalen Rand der letzteren zusammenstösst, dort münden an einer blossen Querspalte die Spinndrüsen aus, ohne sich in irgend einen ansehnlichen Fortsatz auszuziehen. Diese und manche andere Strukturabweichungen wie z. B. das Vorhandensein der Suturlinie auf der Unterlippe und das Getrenntbleiben der Innen- und Aussenladen der Maxille deutet, im Vergleich mit dem der Seidenraupe, auf die letzte, die allgemein ursprünglichere Beschaffenheit des Körperbaues der Blattwespenlarve hin. Die Nahtfurche dehnt sich auch auf den Hypopharynx aus, der, der Innenseite der Unterlippe entsprechend, den weichen Grund der Mundhöhle ausmacht, in welch letztere er polsterartig hineinragt.

Unter allen Mundwerkzeugen der Seidenraupe ist die Unterlippe in ihrem Bau am stärksten differentiiert, d. h., von dem Prototypus am weitesten entfernt, sodass uns ein sehr interessantes Untersuchungsthema dargeboten wird, die Unterlippe der Seidenraupe mit der der primitiveren Blattwespenlarve zu vergleichen. Das Unterkinn (Submentum) der letzteren, das freilich dem Angelglied der Maxille entspricht, besitzt kein Haar, und

das mit dem Haftglied korrespondierende Kinn (Mentum) ist niemals panzerig ausgebildet, sondern von gleicher Festigkeit wie die anderen Teile. Die lateralen Gegenden des Kinns, welche von dem Hauptteil desselben nur unscharf abgegrenzt erscheinen, sind als die Tasterträger (Palpiger) zu deuten, denen ebenfalls kein Haar zukommt. Der Taster (Palpus labialis) setzt sich ebenso aus drei Artikeln zusammen wie der der Seidenraupe, aber darunter bekommt nur der zweite, wie es bei der Maxille auch der Fall ist, ein Haar, während bei der Seidenraupe auch der dritte Artikel nebst dem zweiten, eins davon besitzt. Das Haar des zweiten Artikels springt in der Wespenlarve von beiden Seiten her nach innen vor die Öffnung der Spinndrüsen vor. *Es wäre in seiner Funktion dem Spinnhaar der* Seidenraupe gleichzusetzen, welches dort aber dem Tasterträger angehört. Die Taster der Maxille und der Unterlippe sind voneinander nicht so differentiiert, wie bei der Seidenraupe, sondern weisen fast denselben Gesamtaufbau auf. Bei den Blattwespenlarven stellen die beiden Kauladen (Ligulae od. Zungen) der Unterlippe an jeder Seite je ein einheitliches Gebilde dar. Zwischen den verwachsenen Ligulen der beiden Seiten mündet an deren distalem Rande, wie schon gesagt, die Öffnung der Spinndrüsen median aus. Als die verwachsenen Ligulae wäre in der Seidenraupe das schmale, ringförmige Gebiet um die Basis des Spinnfortsatzes anzusehen. Der häutige Hypopharynx, der die Innenseiten des Kinns und des Unterkinns ausmacht, ist in beiden Arten gut ausgebildet. Die Kehle (Gula), die freilich ein Teil der Kopfkapsel ist, ist in der Blattwespenlarve, den anderen Teilen des Kopfes gegenüber, aus sehr weichem Chitin gebildet. Sie ist durch eine schmale Querplatte dargestellt, die dicht hinter dem Unterkinn liegt, von welchem man sie durch die körnige Beschaffenheit ihrer Oberfläche leicht unterscheiden kann.

III. Der Rumpf.

Die Rumpfsegmente (Abb. 6–8) bestehen aus einer im Querschnitte etwa hufeisenförmigen, festeren Dorsal- und einer flachen, weicheren Ventralplatte von körniger Oberfläche, auf welch letzterer, etwas nach hinten geschoben, ein Paar Brustbeine (im Thorax) oder Abdominalfüsse (im Abdomen) auf beiden Seiten sitzen. Auf jeder Seite der Rückenplatte kommt, ein wenig nach dem vorderen Rand gerückt, je ein winziges Stigma als ein ovaler Schlitz vor, der, etwas dorso-ventral ausgezogen, der allgemeinen Körperringelung parallel angelegt ist. Im letzten Segment des Hinterleibes nimmt die Rückenplatte eine derartige Gestalt an, dass sie wohl mit einem

Viertel der Kugel verglichen werden könnte, und schliesst das Ende des Körpers nach hinten ab. Der vordere Rand des ersten Brustsegmentes ist an der Stelle, wo er sich dicht an die Basis der Unterlippe anschmiegt, stark chitinisiert, an beiden Seiten sogar schwarz gefleckt, um damit die unten verborgene Kehle volkommen gegen die Aussenwelt zu schützen. Sie könnte „Kragen" genannt werden.

Die Brustbeine (Abb. 6) setzen sich aus sechs Gliedern zusammen, welche, abgesehen von dem höchst eigenartigen ersten Glied, von sehr festem, auf der Aussenseite sogar kohlschwarz pigmentiertem Chitin gebildet sind. Die Glieder verjüngen sich distalwärts allmählich, sodass darunter das zweite am kräftigsten entwickelt ist, wogegen das Endglied nur durch eine einzige Kralle dargestellt ist. Die ersten und zweiten Glieder sind auf der latero-frontalen Kante mit einer Längssutur versehen, durch welche die Oberfläche des ringförmig zusammengedrückten ersten Gliedes auf der Aussenseite in einen frontalen, härteren und einen lateralen, weicheren Teil zerlegt ist, von denen der erstere nach der Basis, der letztere nach der Spitze, der Naht entlang, in entgegengesetzter Richtung weit vom ersteren verschoben ist. Bei genauerem Zusehen wird es leicht erkennbar, dass das erste Glied in der Tat aus zwei Ringen von verschiedener Festigkeit besteht. Der weichere Proximalring, der auf der lateralen Seite am breitesten ist, geht nach vorn allmählich aus, während der härtere Distalring an der frontalen Fläche am mächtigsten entwickelt, sogar pigmentiert, an der lateralen dagegen fast nicht mehr zu bemerken ist. Die Brustbeine sind, exklusiv des Endgliedes, reichlich behaart, wie es bei der Seidenraupe auch der Fall ist.

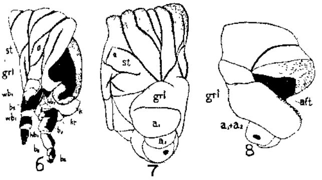


Abb. 6. Die rechte Seite des Prothorax, schief von unten gesehen.

Abb. 7. Das zweiter Abdominalsegment, von der linken, etwas unteren Seite her gesehen.

Abb. 8. Das zehnte Abdominalsegment, von der linken Seite gesehen.

a_1 das erste Glied des Abdominalfusses, a_2 das zweite desselben, aft After, b_2 das zweite Glied des vorderen Brustbeines, b_6 dessen Endglied, grl Grenzlinie zwischen der Rücken- und Bauchplatte, hb_1 härterer Teil des ersten Gliedes des vorderen Brustbeines, k Kehle, kr „Kragen", st Stigma, wb_1 weicherer Teil des ersten Gliedes des vorderen Brustbeines.

Die Abdominalfüsse (Abb. 7) sind sackförmige Ausstülpungen der häutigen Bauchplatte. Sie setzen sich aus zwei halbkugeligen Gliedern zusammen, von denen das erste mit der Basalfläche des Halbkugelgebildes

auf der Bauchplatte, das zweite mit derselben auf dem ersten aufsitzt. Am Scheitel des kleineren zweiten Gliedes, und zwar ein wenig nach der Seite verschoben, ist eine spitze, wie mit einer Nadel gestossene, trichterartige Vertiefung vorhanden, an deren Boden sich von innen ein kräftiges Muskelbündel der Beinmuskulatur ansetzt. Die Einsenkungen könnten als die zeitläufigen Behälter des von den Hautwarzen der Dorsalplatte ausgeschiedenen Schleims gedeutet werden, mit dessen Hilfe die der besonderen Chitinbewaffnung entbehrenden Füsse sich an die Unterlage verkleben zu können scheinen, wobei die feineren Ringelungen der Rückenplatte wohl als die Leitungsrinnen des abfliessenden Schleims dienen könnten. In der Hauptsache aber erfolgt das Festhalten dadurch, dass die Sohlen der beiden Füsse, das Nadelblattstück zwischen sich fassend, gegen einander gepresst werden.

Am letzten Segment des Abdomens (Abb. 8) findest sich der After als eine nach hinten gerichtete Querspalte zwischen der Dorsal- und der Ventralplatte. Die inneren Ränder der Öffnung sind auf beiden, d. i., auf der dorsalen und der ventralen Seite mit zahlreichen, in irgendeinerweise bei der Kotentleerung nützlichen, langen Härchen dicht besetzt. Die Füsse des letzten Segmentes bestehen nur aus einem einzigen Glied. Die Ventralplatte ist bei der Seidenraupe mit dem Fusspaare zusammen gewöhnlich „Kaudalfüsse" genannt, welche aber Herr Prof. Dr. YOSHIMARO TANAKA der Kaiserlichen Universität Kyûsyû als das elfte Abdominalsegment zu deuten versuchte, während die Dorsalplatte in derselben Raupe mit dem Ausdruck „Analplatte" bezeichnet ist. Abgesehen von einer einzigen Querlinie vor dem After, sind bei der Blattwespeularve den anderen Segmenten gegenüber auf der Analplatte keine Abflussrinnen mehr zu erblicken.

Anatomie der Organe.

I. TECHNIK.

Für die Untersuchungen der Organe wurden Schnitte dreier Richtungen, d. i., transversale, horizontale und sagittale in der Dicke von 20μ angefertigt. Die Färbung fand vor dem Schneiden mit Hämalaun statt (Stückfärbung). Auch habe ich Präparate der beiden Körperhälften mit und ohne den Darmtraktus verfertigt, indem der ganze Körper durch einen Sagittal- oder auch Horizontalschnitt halbiert wurde. Färbung wie oben. Für verschiedene Hautgebilde war das Material mit 10 prozentiger Kalilauge behandelt, und zwar in Thermostat über Nacht der Wärme von 50°C

ausgesetzt, und nachträglich zwei Tage in heissem Wasser gelassen, das freilich von Zeit zu Zeit gewechselt wurde, um das Material von der anhaftenden Kalilauge gründlich zu befreien. Spülen mit Brunnenwasser hat dabei arges Schrumpfen hervorgerufen. Chitinpräparate habe ich nie gefärbt.

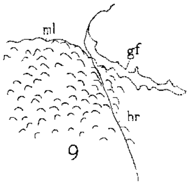
II. Haut und Hautorgane.

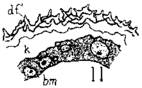
a. Allgemeine Struktur der Haut.

An der Haut der Insekten unterscheidet man drei Hauptschichten; die *Cuticula*, die *Hypodermis* und die *Grundmembran*, von denen die erste wieder aus zwei Schichten besteht, nämlich aus der stark lichtbrechenden Aussenschicht und der lamellösen Innenschicht von weicherem Chitin, welch letztere die Hauptmasse der Cuticula ausmacht (Abb. 10–12).

In der Blattwespenlarve ist die sehr dünne Aussenschicht von den unterliegenden Innenlamellen auf einen Blick zu unterscheiden. Die Hypodermis ist von einer Reihe der Zellen repräsentiert, deren Zellgrenzen völlig verschwunden sind, sodass nur die etwas abgeplatteten Kerne das Vorhandensein einer Zelle verraten. Der Kern ist im Querschnitte oval, in der Flächenansicht kreisrund anzusehen. Gegen den unterliegenden Körperhohlraum ist die Hypodermis von einer sehr dünnen, strukturlosen Membran, der Grundlamelle begrenzt. Die Grenze zwischen der Cuticula und der Hypodermis scheint nicht so ausgeprägt zu sein als allgemein vorgestellt, sondern die eine geht ohne distinkte Grenzlinie in die andere über, sodass in Präparaten, worin beide Schichten häufig von einander getrennt sind, Zickzacklinien entstehen, was ohne Zweifel zu der neueren Ansicht viel beiträgt, dass die Cuticula nicht ausschliesslich von den Hypodermiszellen ausgeschieden wird, sondern sich ein Teil des oberflächlichen Zellplasmas selbst in die Chitindecke verwandelt.

Von der Aussenschicht der Cuticula kommen im allgemeinen verschiedene Skulpturen der Hautoberfläche zustande (Abb. 9–10). In den Blattwespenlarven sind zwei Arten von Fortsätzen zu unterscheiden, namentlich der schüppchenförmige und der dörnchenartige. Die ersteren befinden sich hauptsächlich an der dorsalen Seite der Körperoberfläche, wo die Chitindecke besondere starke Ausbildung aufweist, während die letzteren nur auf die weichere Ventralplatte, einschliesslich der Abdominalfüsse, und auf die ebenfalls weiche Gula des Kopfes beschränkt sind. Die Cuticula der Kopfkapsel, exklusiv der Gula, sowie die der Brustbeine, und zwar bei den letzteren besonders auf der äusseren Seite, ist stark chitinisiert und tritt in





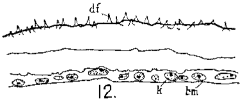


Abb. 9. Die Schüppchenfortsätze der Cuticula, Flächenansicht.

Abb. 10. Quer durch die Cuticula.

Abb. 11. Quer durch die Haut an der latero-ventralen Kante des Abdominalfusses.

Abb. 12. Quer durch die Haut an der Innenfläche desselben.

aus-sch Aussenschicht der Cuticula, bm Basalmembran, df Dörnchenfortsatz mit scharfer Spitze, df' derselbe mit stumpfer Spitze, gf Gelenkfalte, hr hinterer Rand des vorderen Segmentes, in-sch Innenschicht der Cuticula, k Kern der Hypodermiszelle, ml Medianlinie auf der Rückenseite des Körpers, schf Schüppchenfortsatz.

den Chitinpräparaten von den übrigen durchsichtigeren Teilen deutlich hervor. An den genannten Stellen sind die eben beschriebenen Chitingebilde nie anzutreffen. Die schüppchenförmigen Erhebungen der Dorsalfläche scheinen zu der schützenden Tätigkeit der Chitindecke beizutragen, deren Anordnung auf der Rückenplatte etwa mit umgekehrten Dachziegeln verglichen werden könnte, und zwar in dem Sinne, dass jeder Ziegel mit dem freien Rand nach oben, d. i., der Medianlinie des Rückens zugewandt ist.

Wenn auch die Ventralplatte sowie auch die Gula des Kopfes, wie schon beschrieben, mit Dörnchenfortsätzen dicht besetzt sind, so sind dieselben an der latero-ventralen Kante der Abdominalfüsse und auch auf der Innenfläche derselben besonders mächtig entwickelt, ohne aber dass dieselben an beiden Stellen von streng gleicher Gestalt sind (Abb. 11–12). Die Fortsätze der Innenfläche gehen nämlich in eine scharfe Spitze aus, während die an der Kante mehr oder minder abgerundet erscheinen. Zwischen den beiden Sorten der Dörnchenfortsätze aber sind Übergangsformen vorhanden. Sogar die Schüppchenfortsätze möchten von den dörnchenförmigen nie gründlich zu unterscheiden sein, da auch sie in der Tat mit den letzteren mit allenmöglichen Übergängen verbunden zu sein scheinen. Die Fortsätze an der Fusskante wären in gewissem Sinne als eine Zwischenform zwischen denselben auf der Innenseite und den Schüppchenfortsätzen der Rückenplatte zu deuten.

Die Dörnchenfortsätze dürften hauptsächlich dazu dienen, dass von ihnen die Ausscheidung der Hautwarzen auf der Körperoberfläche festgehalten wird, mit der sich die Larve an die Unterlage verklebt. Auch zu der Tastsinneswahrnehmung könnten sie einigermassen nützlich sein.

b. Hautanhänge.

Als Hautanhänge kommen bei den Blattwespenlarven die Haare und die Hautwarzen in Betracht (Abb. 13–14). Beide sind ohne Zweifel von gemeinsamer Herkunft, und befinden sich auf der ganzen Körperoberfläche, und zwar sind die letzteren auf den Rückenschild beschränkt. Sie sind aber sehr spärlich vorhanden und nur vereinzelt hier und da anzutreffen. Ausnahme davon bilden aber der Kopf und die Brustbeine, sowie auch die Umgebung des Afters, wo die Chitindecke deswegen reichlich behaart ist, weil der Kopf beim Fortbewegen vorangeht und mit der Aussenwelt vorerst in Berührung kommt, und weil die Brustbeine bei der Nahrungsaufnahme zum Spülen und Festhalten der Futterblätter benutzt werden, und endlich weil durch den After Kot ausgeschieden wird. Kurz ausgedrückt, *stellen all die genannten Gegenden die* Regionen dar, wo die Tastempfindung besonders beansprucht wird; die Haare scheinen mehr oder minder mit der Tastempfindung in Beziehung zu stehen. Dass zur Tastsinneswahrnehmung bei der Nahrungsaufnahme und auch bei der Ausscheidung der Spinnfäden die Aussenfläche des Labrums sowie die Palpen der beiden Maxillen mit besonders langen Haaren versehen sind, ist schon bei der Beschreibung des Kopfes erwähnt worden.

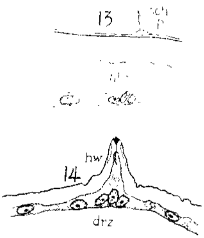


Abb. 13. Quer durch die Haut, wo sich ein Haar befindet.
Abb. 14. Quer durch die Haut, wo sich eine Hautwarze befindet.
drz Drüsenzelle, hbz Haarbildungszelle, hw Hautwarze, p Haarpapille, sch Haarschaft.

Der Hautteil des Haares, der Haarschaft, sitzt gelenkig auf einer runden Aushöhlung der Haarpapille, d. i. auf der Alveole auf. Die Haarpapille ist eine kuppelige Hauterhebung, unter welcher gewöhnlich zwei Haarbildungszellen vorkommen, die ohne Zweiful von den Hypodermiszellen differentiiert sind.

Die Hautwarzen, von denen der Schleim ausgeschieden wird, lassen sich leicht von den gewöhnlichen Haaren ableiten. Sie sind nichts anders als ein umgeformtes Haar, dem der Schaft durch Degeneration verloren gegangen ist. Der winzige Zapfen auf der Warzenspitze, an welchem die Drüse ausmündet, möchte wohl die Rudimente des rückgebildeten Schaftes sein. Auch wären die Drüsenzellen ohne weiteres als umgewandelte Haarbildungszellen zu deuten. Die Papille hat sich hier zu einem hohen Kegel ent-

wickelt, welcher den Warzenkörper ausmacht, unter dem die Drüsenzellen liegen. Von denselben Zellen führt ein haardünner Kanal zu dem Zäpfchen. An der Spitze der Warze hat sich schwärzlicher Farbstoff angesammelt.

III. Sinnesorgane.

a. Hautsinnesorgane.

Auch hier sind, wie in der Seidenraupe, die Hautsinnesorgane neben den Sinneskegeln von verschiedenen Geruchsgruben vertreten, welche, je nach der Gegend, worin sie sich befinden, von mehr oder minder voneinander abweichender Beschaffenheit zu sein scheinen. Sie beschränken sich bei der Blattwespenlarve auf die Kopfkapsel, die Antennen, die Mundwerkzeuge und die Aussenflächen der Brustbeine. Die der Kopfkapsel (Abb. 15) kommen in der Mehrzahl fast in einer Reihe angeordnet zwischen der Antenne und dem Oberkiefer vor. Sie sind verschieden gross, aber alle von einer distinkten Kreislinie von der übrigen Cuticula abgesetzt, innerhalb der ein dünnes Chitinhäutchen ausgespannt zu sein scheint. Sie seien gewöhnliche Sinnesgruben (Sensillum placodeum mit dünner Platte) genannt.

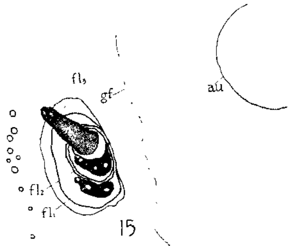


Abb. 15. Sinnesorgane um und an der Antenne.
au Auge, fl_1 erstes Glied des Fühlers, fl_2 zweites Glied desselben, fl_3 drittes Glied desselben, gf Rand des Genalflecks.

Die Antenne trägt vier Sorten von Gruben (Abb. 15). Auf dem ersten und zweiten Glied sind beziehungsweise vier und fünf Gruben von verschiedener Grösse vorhanden, *von denen je zwei so gigantisch* entwickelt sind, dass sie selbst bei Lupenvergrösserung leicht aufgefunden werden können. Die kleinste ist auf jedem Glied von einem in der Mitte mit einem Grubenkegel versehenen Exemplar dargestellt. Abgesehen von den genannten zwei Sorten sind alle übrigen gewöhnliche Sinnesgruben. Die gesamten Gruben der beiden Glieder sind gemeinsam von einem scharzen Fleck umgeben, welcher bei der Sinneswahrnehmung den Grubenorganen in irgendeinerweise Beistand leisten könnte. Das dritte

Glied ist am distalen Ende mit vier Sinnesgruben ausgestattet, welche aber nicht so deutlich hervortreten wie die der ersten zwei Glieder. Mutmasslich sind sie das sogenannte Sensillum placoideum, das mit einer dicken Sinnesplatte ausgerüstet ist.

Die Oberlippe (Abb. 16) hat auf der Unterseite jederseits eine Reihe nach der Mittellinie zeigender Sinneskegel sowie auch zerstreute Gruppen der gewöhnlichen Sinnesgruben. Am distalen Rande derselben reihen sich zahlreiche, wohlentwickelte Kegelgruben (Sensillum coeloconicum) nebeneinander, welche aber auch auf der Oberseite vereinzelt aufgefunden werden können. Die grösseren Kegel sind mit einem gegabelten Ende versehen. Wie schon beschrieben, sind Sinneskegel auch am Rand der Innenlade der Maxille vorhanden. Die Sinneskegel, sogar auch die Sinnesgruben könnten sich von einem gewöhnlichen Haare ableiten lassen. *Die Funktion der ersteren ist* nicht klar zu beweisen; der Lage nach werden sie aber nebst der Tastempfindung vermutlich auch der Geruchs- oder sogar der Geschmackswahrnehmung dienen.

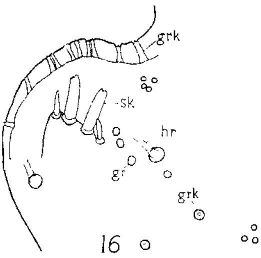


Abb. 16. Unterseite der rechten Hälfte des Labrums.
gr Sinnesgrube ohne Kegel, grk dieselbe mit Kegel, hr Haar, k Sinneskegel. (Die mit Punktlinien gezeichneten Gebilde finden sich auf der Oberseite des Labrums.

Zahlreiche Geruchsgruben sind ferner auf jedem Brustbeine vorhanden (Abb. 17–18), und zwar auf der Aussenseite jedes Gliedes, abgesehen von dem krallenförmigen Endglied, besonders reichlich aber auf dem vierten, sodann auch auf dem fünften Glied. Sie scheinen von wesentlich gleicher Beschaffenheit wie die an der Antennenspitze zu sein, d. h., der Raum ihrer Grube erscheint nicht so vollkommen durchsichtig wie der der gewöhn-

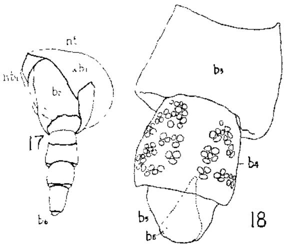


Abb. 17. Hinteres Brustbein, von der Seite gesehen.
Abb. 18. Geruchsgruben auf dem vierten Gliede desselben Beines.
b_2-b_6 Glieder des Beines, hbl härterer Teil des ersten Gliedes, nt Naht auf dem ersten und dem zweiten Gliede, ubl weicherer Teil des ersten Gliedes.

lichen auf der Kopfkapsel und auf den ersten zwei Gliedern der Antenne, sondern bleibt vielmehr leicht trüb nuanciert. Also dürften sie vielleicht ein Sensillum placoideum mit dickerer Sinnesplatte sein. Demzufoge, dass sie in Gruppen vorkommen, in denen mehrere gegeneinander dichtgedrängt liegen, weichen sie dazu noch in Gestalt von den übrigen ziemlich ab, indem sie häufig polygonale, meist hexagonale oder pentagonale Formen anzunehmen geneigt sind.

b. Lichtsinnesorgane.

Wie beschrieben, kommt in der Blattwespenlarve als Lichtsinnesorgane statt Gruppen derselben, ein einziges Paar mächtig entwickelte Punktaugen vor.

IV. Körpermuskulatur.

a. Muskeln der Körpermuskulatur.

Die Muskelfaser (Abb. 19–20) ist mit einer peripherischen Hüllhaut, dem Sarcolemma, überzogen, welche den gesamten Inhalt der Faser umschliesst. Dieser besteht aus dem Sarcoplasma, in welches die Kerne und die kontraktilen, quergestreiften Myofibrillen derart eingelagert sind, dass im Zentrum der von Fibrillen ausgefüllten Faser ein Säul-

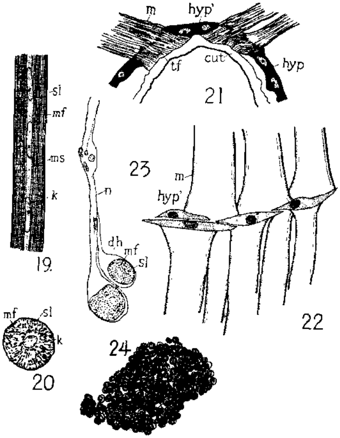


Abb. 19. Muskelfaser.
Abb. 20. Dieselbe quer.
Abb. 21. Transversaler Schnitt durch die Bauchplatte an der Stelle zwischen den beiden Füssen eines Fusspaares der Abdominalfüsse.
Abb. 22. Ansätze der Längsmuskeln (Musculus longitudinalis sterni) an einer Segmentgrenze der ventralen Haut.
Abb. 23. Nervenendigungen an den Muskelfasern.
Abb. 24. Anlage des imaginären Muskels.
cut Cuticula, dh Doyèrescher Hügel, hyp Hypodermis, hyp' dieselbe zwischen den Muskelansätzen, k Kern der Muskelfaser, m Muskelfaser, mf Myofibrillen, ms Myosarc, n Nerv, sl Sarcolemma, tf Tonfibrillen.

chen von fibrillenfreiem Myoplasma (Myosarc) gebildet ist, in welchem die zahlreichen, längsausgezogenen Kerne der Achse entlang in einer Reihe mit den davon freien Myoplasmateilchen alternieren.

Die Muskelfaser setzt sich mit den beiden Enden an das Chitinskelett an. Der Ansatz findet durch Vermittlung einer von den Epidermiszellen verwandelten Sehne statt, welche die sogenannten Tonfibrillen enthält (Abb. 21–22).

Die motorischen Nerven werden durch eine Endplatte, den sogenannten DOYÈREschen Hügel, an die Muskeln angeknüpft. Der Hügel liegt auf einer Seite der Muskelfaser auf, wobei sich die Substanz der Endplatte unmittelbar in das Sarcolemma fortsetzt, sodass die Muskelfaser im Querschnitte von derselben Platte umgriffen erscheint (Abb. 23).

b. ANLAGE DER IMAGINÄREN MUSKELN.

Im Körperinnern der Blattwespenlarve werden, zu beiden Seiten ungefähr symmetrisch verteilt, eine Anzahl Zellmassen von rundlichem Umriss gefunden, welche von zahlreichen, winzigen, gegeneinander dichtgedrängten, in einer gemeinsamen, homogenen Grundsubstanz eingebetteten Zellen zusammengesetzt sind (Abb. 24). Diese sind die Muskelbildungszellen, zwischen denen sich später die imaginären Muskelfasern ausbilden sollen.

Sie sind etwa den Leucocyten ähnlich; sogar wären sie von denselben abzuleiten. Nur sind sie kleiner als die letzteren, die homogene Plasmaschicht erscheint etwas dunkler und dünner und der Kern reicher an Chromatin. Auch die Leucocyten bilden, wie unten zu besprechen, gelegentlich Zellmassen, von denen aber kein distinkter Umriss nachzuweisen ist, sodass sie leicht von den Muskelanlagen unterschieden werden, mit welchen sie vielleicht verwechselt werden könnten.

V. DER DARMTRAKTUS.

a. DARMEPITHEL.

Wie in der Seidenraupe, so ist auch hier der Darmtraktus zum grössten Teil vom Mitteldarm eingenommen, der, in der Körperachse gelegen, den Rumpf geradlinig von vorn nach hinten durchzieht und den Hauptteil der gesamten Eingeweide ausmacht. Sein Epithel ist von einer Reihe von Nährzellen gebildet, welche der von ihnen ausgeschiedenen dünnen, strukturlosen Grundmembran, der Membrana propria, aufliegen. Es sind drei

Sorten von Nährzellen vorhanden, welche vorläufig *Zylinder-*, Becher- und Keilzellen genannt seien, ohne doch denselben Zellen anderer Insekten genau zu entsprechen. Sie verhalten sich vielmehr ziemlich anders als die letzteren. Die Keilzellen sind indessen reine Regenerationszellen, die vereinzelt zwischen den Basen anderer Nährzellen aufgefunden werden, während die Zylinder- und Becherzellen aller Wahrscheinlichkeit nach nur verschiedene Stadien desselben Zellzyklus, und zwar die ersteren das ältere und die letzteren das jüngere Stadium desselben darzustellen scheinen (Abb. 25–28).

Die Zelloberfläche des Darmepithels ist, wie bei den meisten Insekten, in einen Stäbchensaum, das Rhabdorium, differentiiert, welches aus zu der Oberfläche senkrecht stehenden, dicht nebeneinander gedrängten, feinen Fädchen von unbeweglicher Natur zusammengesetzt ist. Die Frage nach der Bedeutung des Stäbchensaumes ist verschieden beantwortet worden. Nach den herrschenden Ansichten vieler Autoren wäre ihm aber eine schützende Rolle zuzuschreiben, und zwar in dem Sinne, dass von ihm, bei der Tätigkeit der Nährzellen, diesen die ungestörte, gleichmässige Ausscheidung oder auch Aufsaugung des Sekrets bezüglicherweise der Nährlösung gewährleistet werde.

Das Rabdorium ist an ihrer Seite wieder mit einem zarten Häutchen, der *peritrophischen Membran, Membrana anista,* überzogen, welche unzweifelhaft von den Nährzellen selbst gebildet worden ist. Seine Funktion ist nicht klar bekannt. Da es aber nur an den Zylinderzellen vorkommt, so dünkt es mich sehr wahrscheinlich, dass von ihm das zarte Rabdorium mechanisch gegen den Darminhalt geschützt werde. Die Membran liegt dicht dem Stäbchensaum auf. Sie ist an den älteren Zellen stärker entwickelt, wird bei der Degeneration der Zellen (siehe unten) vor dem Zerfall des Zellkörpers in das Darmlumen abgeworfen, wo mehrere davon, dicht über einander gelegt, noch längere Zeit erhalten bleiben, weil sie von einer sehr widerstandsfähigen Substanz gebildet zu sein scheint. Also kommt eine Schicht der peritrophischen Membranen zustande, welche im Darmlumen überall dem Epithelium entlang und zwar im gleichen Abstand von demselben aufgefunden werden.

Gelegentlich wird in den Zylinderzellen, und zwar denen des jüngeren Stadiums, unter dem vorhandenen Rhabdorium ein neues gebildet, wobei das ältere sich zusammen mit der Membrana anista von der Zelloberfläche ablöst, sodass in der eben beschriebenen peritrophischen Schicht auch Rhabdorien zwischen einzelnen Membranen vorkommen können (Abb. 29).

Bei den Keilzellen fallen all die genannten peripherischen Differentiierungen aus, da sie gar keine Innenfläche haben. Sie sind, mit der

breiteren Basis auf der Membrana propria sitzend, vollkommen zwischen den Zylinderzellen eingeschaltet, sodass das spitze Ende niemals zur Oberfläche des Epithelium kommt. Die Zellen sind in der Tat von recht unbedeutender Grösse, und befinden sich nur vereinzelt zwischen den Basis der Darmepithelzellen; sie könnten leicht übersehen werden. Allmählich wachsen sie aber in die Breite, und zwar in der Masse, dass die nun von zahlreichen, parallelen, zu der Oberfläche senkrechten Streifen durchzogenen, proximalen Teile der alternden Zylinderzellen, von ihnen verdrängt, nach und nach magerer werden, bis dass aus den Keilzellen zum Schluss stumpfe Kegelgebilde zustandekommen, in welchen bereits je eine mit Sekret gefüllte Vakuole sichtbar ist. Beim genaueren Zusehen wird es sich wohl verraten, dass die Vakuole mit einer dünnen Schicht ausgekleidet ist, in welcher schon das Rhabdorium der fertigen Zelle vorgebildet wurde.

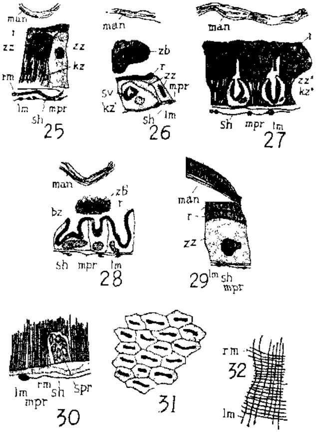


Abb. 25–29. Transversaler Schnitt durch die Darmwand.

Abb. 30. Transversaler Schnitt durch einen Epithelialspross. (Nur die äusseren Schichten der Darmwand dargelegt.)

Abb. 31. Flächenansicht des Darmepithels, das ausschliesslich aus Zylinderzellen besteht.

Abb. 32. Gitterwerk der Darmwandmuskeln, Flächenansicht. bz Becherzelle, kz kz′ kz″ Keilzellen, lm Längsmuskel, man Membrana anista, mpr Membrana propria, r Rhabdorium, rm Ringmuskel, sh seröse Hülle, spr Epithelialspross, sv Sekretvakuole, zb zb′ Zerfallsballen, zz zz′ zz″ Zylinderzellen.

Der Kern ist bis zu dieser Zeit durch ein winziges, kreisrundes Gebilde an der Zellbasis dargestellt, welches, mit spärlichem Chromatin versehen, auch in seinem Inhalt manche jugendliche Beschaffenheiten aufweist. Nunmehr gewinnt es aber beträchtlich an Masse, und zu gleicher Zeit wird er von zahlreichen feineren Chromatinkörnchen dicht ausgefüllt. In dem ausgebildeten Zustand kommt er sogar dazu, eine zu der Transversalebene parallel abgeplattete Form anzunehmen, womit freilich eine viel breitere Reaktionsfläche gegen das Zytoplasma geschafft wird, was

ohne Zweifel dahin deutet, dass die Zellaktivität nun lebhaft vor sich geht (Abb. 31).

Indessen schreitet das Wachstum der Kegelzelle unaufhörlich fort. Der Inhalt der nun ansehnlich gewachsenen Sekretvakuole, von welcher der grösste Teil des Zellkörpers eingenommen ist, wird dadurch in das Darmlumen entleert, dass die Plasmawand um die Vakuole an dem peripheren Ende der Zelle ausbricht. An dem so entstandenen Loch weichen nun die Ränder voneinander ab, wodurch zuletzt die Becherzelle zustandekommt. *Zu dieser Zeit ist der basale Teil der degenerierenden Zylinderzelle* völlig verschwunden. Auch der den Kern umschliessende Hauptteil am inneren Ende gerät, nachdem er die Membrana anista abgeworfen hat, zusammen mit dem Rhabdorium schliesslich in Zerfall, indem der Kern, das Plasma und das Rhabdorium, gleichzeitig vakuolisiert, gemeinsam in einen grossen, rundlichen, homogenen Haufen, den Zerfallsballen, übergehen, worin die genannten Partien am Ende voneinander ununterscheidbar werden, um sich zuletzt auf der Stelle vollständig aufzulösen.

Der Hohlraum der von der Sekretvakuole entstandenen Einbuchtung der Becherzelle wird nun von dem anschwellenden Plasma der Zellbasis allmählich von unten verdrängt, sodass die Zelle Schritt für Schritt in die Zylinderzelle übergeht, von welcher, wie beschrieben, die Membrana anista für das nun direkt dem Darminhalt ausgesetzte Rhabdorium neu gebildet wird. In den jüngeren Zylinderzellen sind die parallelen Streifen am basalen Ende nur recht undeutlich ausgebildet.

Die jungen Becherzellen sind anfänglich noch mit der Reihe der degenerierenden Haufen der Zylinderzellen überzogen. Sonst sind die Sekretkugeln *im* DEEGENER*schen Sinne nirgends in dem Darmlumen aufzufinden.* Mir scheint die Sekretion bei der vorliegenden Wespenlarve auf zweierlei Weisen vor sich zu gehen, nämlich erst durch die Entleerung der Sekretvakuole bei der Bildung der Becherzellen, und dann auch durch das Auflösen des Zellkörpers selbst bei der Degeneration der Zylinderzellen, wobei nicht Sekretkugeln abgestossen, sondern erst die genannten Zerfallsballen gebildet werden. Die letzteren lösen sich am Ende vollkommen auf, sodass wenn die neuen Zylinderzellen vollständing ausgebildet werden, keine Spur von den Ballen dort übrig bleibt. Den Zylinderzellen ausschliesslich scheint mir die Resorbtionstätigkeit znzukommen, währeud die Becherzellen bloss als Zellen in Uebergangsstadien aufgefasst werden mögen.

Gelegentlich kommt auch eine kleine Gruppe winziger Zellen, der sogenannte Epithelialspross, als Regenerationszellen vor, die, im Plasma einer jüngeren Zylinderzelle eingeschlossen, auf der Basalmembran aufsitzt

(Abb. 30). Ihr Kern erinnert uns in Grösse, Form und Beschaffenheit sehr an den der Keilzellen. Die Entfaltung des Sprosses habe ich aber nicht verfolgen können, betreffs deren eingehenderer Mitteilungen der Leser auf meine weiteren Arbeiten verwiesen sei.

b. DIE ENTOPLEURA.

Als Entopleura kommen zwei Muskelschichten vor, welche von aussen den Darmtraktus umhüllen. Die innere Schicht besteht im Mitteldarm aus Ring-, die äussere aus Längsmuskeln. Die Muskelfassern der beiden Schichten stehen, wenigstens im Mitteldarm, keineswegs mit einander in Beziehung. Von ihnen kommt ein regelmässiges Gitterwerk um den Darmkanal zustande, worin sich die Muskeln der beiden Sorten, in gleichmässigen Zwischenräumen, immer fast rechtwinkelig kreuzen (Abb. 32).

Ausser den genannten Muskellagern kommt noch dazu eine strukturlose, seröse Hülle, das Peritoneum, vor, welche als die äusserste Schicht der Darmwand die Muskelfasern, wenn auch nicht lückenlos, nach aussen umschliesst, mit welch letzteren, und zwar mit den Längsfasern, sie häufig verwachsen ist.

Ob sie aus embryonalen Bindegewebszellen hervorgeht, die sich aneinander lagern und unter Verlust der Kerne zu einem hyalinen Strange ausziehen, ist nicht sicher ermittelt. MACDUNNOUGH fand bei *Chrysopa* (Larve und Imago) eine seröse Hülle am Mitteldarm, welche sich zwischen die innere Ringsmuskelschicht und die äussere Längsmuskellage einschiebt. Er sah sie auch am Dünndarm der *Chrysopa* Imago zwischen Muskulatur und Basalmembran. VERSON verneinte das Vorhandensein des besonderen Peritoneums und setzte die seröse Hülle anderer Autoren dem Sarcolemma gleich.

VI. SPINNDRÜSEN.

Die Spinndrüsen der Blattwespenlarve bestehen, wie die der Seidenraupe, aus ein Paar langer Schläuche, welche kurz vor der Ausmündung an dem Labrum sich zu einem unpaaren Ausführungsgang vereinigen. Der Schlauch mag auf jeder Seite in zwei Abschnitte zerlegt werden, von denen der vordere, gerade nach vorn und innen verlaufende Teil von mehreren acinösen Drüsen besetzt ist (Abb. 33), während der hintere Abschnitt, der den Hauptteil des gesamten Drüsensystems ausmacht, stark verwunden, dem Darmkanal von aussen anliegt. Er ist mit zahlreichen einzelligen

Drüsen ausgestattet. Der Schlauch und die Drüsenzellen liegen alle in einer Zylinderfläche um den Darmkanal, auf der die Zellen parallel zu beiden Seiten des Schlauches in einer Reihe angeordnet sind (Abb. 36).

Die einzelligen Drüsen funktionieren ohne Zweifel als die eigentlichen Spinndrüsen, während den acinösen Drüsen des vorderen Abschnittes eine Schleimsekretion zuzuschreiben wäre. Die letzteren mögen den nur in einem Paar vorkommenden FILIPPIschen Drüsen der Seidenraupe gleichgesetzt werden, deren Funktion noch nicht sicher bekannt ist.

Die Wände der verzweigten Ausführungsgänge einzelner acinösen Drüsen sind ganz von derselben Beschaffenheit wie die der Hauptschläuche, welche in ihrer ganzen Länge wieder fast auf gleiche Weise gebaut sind. Auch die sogenannten mittleren Teile der Seidenraupendrüsen lassen sich hier nicht nachweisen. Die Schläuche des vorderen Abschnittes werden bei der Wespenlarve nach vorn allmählich dünner.

Der grundliegende Unterschied der Spinndrüsen zwischen den Larven der Blattwespen und den Seidenraupen scheint wohl darin zu suchen zu sein, dass in der letzteren das Seidenmaterial von den Wandzellen selbst ausgeschieden wird, wogegen bei den ersteren die Schläuche dafür mit besonderen, der Schlauchwand ansitzenden, einzelligen Drüsen ausgerüstet sind. Bei ihnen ist in den Wandzellen keine sekretorische Struktur zu beweisen. Wie unten beschrieben ist, sind die Zellen vielmehr sogar der Degeneration zugeneigt. Indessen könnten die Drüsenzellen in gewissem Sinne als differentiierte Wandzellen interpretiert werden. Sie sind durch Vermittlung einer Zelle (der Halszelle) mit dem Wandepithelium direkt verbunden (Abb. 37).

In den acinösen sowie auch in den eigentlichen Drüsen sind die sekretführenden Hohlräume intrazelluläre Vakuolen, von denen das Plasma fast ausgefüllt ist (Abb. 34–35, 37–38). Der Kern ist in beiden Drüsen anfänglich von einem ziemlich grossen, mit mehreren Nukleolen und zahlreichen Chromatinkörnchen versehenen, rundlichen

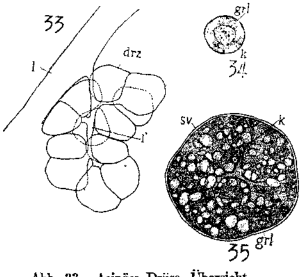


Abb. 33. Acinöse Drüse, Übersicht.
Abb. 34. Jüngere Zelle der acinösen Drüse.
Abb. 35. Ältere Zelle der acinösen Drüse.
drz Drüsenzelle, grl Grundlamelle, k Kern der Drüsenzelle, l Leiterschlauch der gesamten Spinndrüsen, l' Leiter der acinösen Drüse, sv Sekretvakuole.

Gebilde dargestellt. *Mit der Zeit* aber vollzieht sich in ihm eine allmähliche aber weitgehende Veränderung sowohl in der Form als auch in den Beschaffenheiten, sodass in den älteren Zellen ein stark ramifizierter, von den Sekretvakuolen zusammengedrückter, magerer Kern zustande kommt, in welchem nun die Nukleolen völlig verschwunden, die Chromatinkörnchen aber noch feiner und zahlreicher geworden sind. Die Peripherie der Drüsenzelle ist in eine Grundlamelle differentiiert.

Die Art und Weise wie die Zellen der eigentlichen Drüsen mit dem Schlauch verbunden werden, ist höchst bemerkenswert (Abb. 37–39), insoweit sich zwischen die Drüsenzelle und das Schlauchepithel anfangs eine solide Halszelle einschiebt. Die Halszelle ist unzweifelhaft eine umgewandelte Epithelzelle des Schlauches, da sie mit einem Ende direkt an das Schlauchlumen angrenzt.

Im Plasma der Drüsenzelle entsteht nun an der Stelle, wo sie mit der Halszelle in Verbindung steht, eine kleine Vakuole, welche Halshöhle genannt werden könnte. Die Höhle dehnt sich allmählich aus, dringt sogar in das Plasma der Halszelle hinein, bis schliesslich die Zelle von ihr vollkommen ausgefüllt wird. Also kommt die Halshöhle, welche nun besser Halskanal genannt sei, in direkte Verbindung mit dem Lumen des Schlauches, und der von dem Kanal verdrängte Kern der Halszelle befindet sich jetzt ganz verkleinert an derselben Stelle der Wand, wo das Schlauchlumen in den Halskanal übergeht.

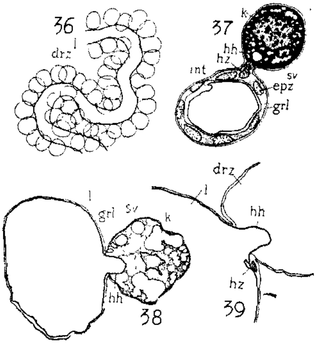


Abb. 36. Ein Teil des hinteren Abschnittes der Spinndrüse, Flächenansicht.
Abb. 37. Quer durch den Spinnleiterschlauch im hinteren Abschnitt, mit einer jüngeren Drüsenzelle.
Abb. 38. Quer durch denselben mit einer älteren Zelle.
Abb. 39. Quer durch denselben wie in Abb. 38, nur die Halsgegend dargelegt.
epz Epithelzelle des Schlauches, hh Halshöhle, hz Halszelle, int Intima, weitere Bezeichnungen wie in Abb. 33–35.

Das Epithel des Schlauches besteht im jüngeren Stadium aus einer Reihe der den Hypodermiszellen sehr ähnlichen Pflasterzellen, welche basal einer Grundlamelle aufliegen und oberflächlich eine wohl entwickelte Intima haben (Abb. 37). Die

Epithelzellen sind aber der Degeneration zugeneigt, sodass das Schlauchlumen im fertigen Zustande von einer dünnen, fast kernlosen, stark lichtbrechenden, chitinösen Membran begrenzt ist (Abb. 38).

In dem gesamten System der Spinndrüsen befinden sich die Drüsenzellen der beiden Arten, sowie auch die Zellen des Schlauchepithels in den hinteren Teilen am weitesten entwickelt, in den vorderen dagegen noch in ganz jüngeren Stadien der Entwicklung. Vor allem sind die vordersten der acinösen Drüsen stets von jüngsten Zellen winziger Grösse vertreten. Also könnten die Spinndrüsen in ihrer Entfaltung wohl mit einem herabsteigenden Blütenstand verglichen werden. Im ganzen genommen aber herrschen in den jüngeren Individuen freilich jüngere Zellen, während bei den älteren Larven alle Drüsenzellen in einem mehr oder minder fortgeschrittenen Zustand vorkommen.

Endlich sei bemerkt, dass selbst zwischen den beiden Sorten der Drüsenzellen alle möglichen Übergänge, wenigstens in äusserer Gestalt, vorhanden zu sein scheinen. Die eigentlichen Drüsen könnten in dieser Beziehung als eine acinöse Drüse mit einer einzigen Zelle aufgefasst werden. In der Tat gibt es viele der acinösen Drüsen, welche nur mit wenigen, gelegentlich sogar mit zwei oder auch nur einer Zelle ausgerüstet sind. Die Beschaffenheiten des Zellplasmas aber lassen sich zwischen den beiden Sorten ziemlich scharf unterscheiden, indem dasselbe der acinösen Drüsen mit zahlreicheren, kleineren Sekretvakuolen versehen ist (Abb. 35), während in den eigentlichen Drüsen die letzteren, wenn auch meistens in geringerer Anzahl, zur mächtigen Grösse entwickelt sein können. Dort pflegen sie mehrere nebeneinander in einer Reihe angelegt vorzukommen, sodass in den jüngeren Zellen häufig rosenkranzartige Gestalten entstehen, von denen der Kern allerseits umgeben ist (Abb. 37).

VII. Malpighische Schläuche.

Die Blattwespenlarve besitzt 24 malpighische Schläuche, welche jeder für sich und auf gleicher Höhe in gleichmässigen Abständen von einander an der Grenze zwischen dem Mittel- und Enddarm, und zwar auf der Seite des ersteren in das Darmlumen einmünden (Abb. 41). Sie verlaufen, in Schlingen gelegt, am Darm erst eine kurze Strecke nach vorwärts und dann rückwärts, um schliesslich am Rectum in blinden Enden frei zu terminieren, statt, wie bei der Seidenraupe, zwischen die Schichten desselben einzudringen. Am Ende ist stets ein Kern nachzuweisen, der wohl als derselbe einer Schliesszelle gedeutet werden kann (Abb. 42).

Gelegentlich sei hier bemerkt, dass der Enddarm sich, wie bei den Seidenraupen, aus vier Teilen zusammensetzt (Abb. 40), nämlich dem Dünndarm, dem ersten und dem zweiten Dickdarm und dem Rectum, von denen jeder in seiner relativen Lage und auch in seiner Struktur mit dem entsprechenden Teile des Seidenraupendarms in wesentlichen Punkten fast völlig übereinstimmt.

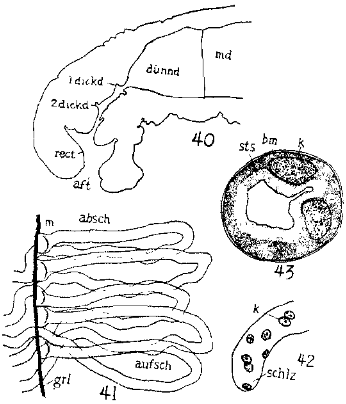


Abb. 40. Hinterer Teil des Darms, Seitenansicht.
Abb. 41. Anfangsteil der malpighischen Schläuche, von denen nur fünf abgebildet sind.
Abb. 42. Endteil eines Schlauchs.
Abb. 43. Quer durch den Schlauch.
absch absteigender Schenkel, aft After, aufsch aufsteigender Schenkel, bm Basalmembran, dickd Dickdarm, dünnd Dünndarm, grl Grenzlinie zwischen dem Mittel- und dem Enddarm, k Kern der Schlauchwandzellen, m Mündung des Schlauchs, md Mitteldarm, rect Rectum, schlz Kern der Schliesszelle, sts Stäbchensaum.

Die Schläuche haben in ihrer ganzen Länge ungefähr denselben Durchmesser und auch denselben histologischen Aufbau (Abb. 43). Das Lumen ist nämlich von einem grosszelligen Epithel ausgekleidet, dessen kernhaltiger Teil häufiger in das Lumen hineinragt. Der Kern ist gross, feinkörnig und von einem rundlichen Umriss. Das Epithel liegt auf einer Basalmembran und trägt an seiner Oberfläche einen Stäbchensaum. Die sogenannte Peritonealhülle mancher Autoren ist nie zu beweisen. Der Wespenlarve wenigstens fehlt sie ohne Zweifel. In dem Epithel ist keine Zellgrenze vorhanden, sodass es sich hier um ein Syncytium handelt. Um den Schlauch ist ferner keine Muskelschicht zu bemerken.

VIII. Nervensystem.

Das Bauchganglion setzt sich, wie es bei den meisten Insekten der Fall ist, aus einem Paar verwachsenen Ganglien zusammen. Auch die

beiden Konnektiven bleiben rückwärts von jedem Ganglion noch eine Strecke mit einander zusammengewachsen. Ein Nerv läuft auf beiden Seiten vom Ganglion aus, in welch letzteres hinter dem Nervenausläufer ein Tracheenast hineintritt. Von den Tracheen kommt ein anderer Ast der einen Seite unter dem Ganglion mit demselben der anderen Seite in Verbindung, jedoch ohne dass eine membranöse Portion dazwischen eingeschaltet ist (Abb. 44).

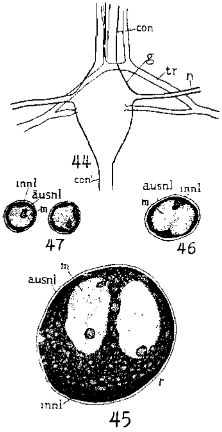


Abb. 44. Bauchganglion, von oben gesehen.
Abb. 45. Quer durch das Bauchganglion.
Abb. 46. Quer durch den zusammengewachsenen Teil der Konnektiven.
Abb. 47. Quer durch die Konnektiven.
äusnl äusseres Neurilemm, con Konnektiven, con′ verwachsener Teil derselben, g Ganglion, innl inneres Neurilemm, m Markschicht, n Nerv, r Rindenschicht, tr Tracheenast.

Im Querschnitte (Abb. 45) sind an dem Ganglion Schichten verschiedener histologischer Strukturen zu unterscheiden. Von aussen nach innen aufzuzählen, sind sie nämlich das spärlich mit länglichen Kernen versehene, dünne, membranöse, durchsichtige äussere Neurilemm, das granulär zellige, ebenso kernarme innere Neurilemm, die Rindenschicht mit zahlreichen meist rundlichen Ganglienzellen, und im innersten die Markschicht, die sogenannte Punktsubstanz, wo die Nervenfibrillen verlaufen. Die Kerne der beiden äusseren Schichten sind fast von derselben Beschaffenheit. Da die Ganglienzellen der Rindenschicht, besonders die der peripheren Partien, häufig von länglicher Gestalt sind, so können sie mit den Zellen des inneren Neurilemms leicht verwechselt werden. In der Tat ist zwischen den beiden Schichten keine scharfe Grenze wahrzunehmen. Die Rindenschicht ist an der ventralen Seite am stärksten entwickelt. Median keilt sie auf der dorsalen und auch auf der ventralen Fläche tief in die Markschicht ein. Die Ganglienzellen sind von verschiedener Grösse. Häufig kommen unter ihnen mächtige Zellen vor. Sie sind vereinzelt auch in der Markschicht anzutreffen. In den Konnektiven (Abb. 46–47), und freilich auch in allen Nervenfasern kommt die Rindenschicht nicht vor. Die Zellen in der Markschicht könnten wohl bindegewebige Elemente sein.

IX. Respirationsorgane.

Ein starker Tracheenlängsstamm läuft auf jeder Seite der Körperoberfläche entlang, auf welcher sich die Stigmata, in der Regel je ein Stigma auf einer Seite jedes Segmentes, befinden (Abb. 48–49). Das Stigma steht durch ein kurzes Rohr mit dem Stamm in Verbindung. Es ist durch einen der Körperringelung parallelen Schlitz dargestellt, welcher von einem Chitinring umgeben ist, der ihn offen hält. Nach innen führt von dem Stigma ein im Querschnitte elliptischer Hohlraum, der Anfangsteil des soeben besprochenen Verbindungskanals, dessen Wand mit zahlreichen Haaren besetzt ist. Am Boden des Hohlraums erhebt sich nun eine trichterförmige Membran, welche in der Mitte eine schmale, längliche Öffnung frei lässt. Da die Membran aus zwei Schichten dünner Chitinhäutchen aufgebaut ist, so mag sie ohne Zweifel als eine Faltenbildung des Kanalwandchitinüberzuges aufgefasst werden. Die Membran ist ferner auf ihrer Aussenseite von zahlreichen, langen, verfilzten und verzweigten Haaren gestützt, deren distale Enden mit der Membran verwachsen sind. Die Haare sitzen auf der Wand des Hohlraumes. Sie repräsentieren also die innersten Haare der allgemeinen Behaarung des genannten Raumes.

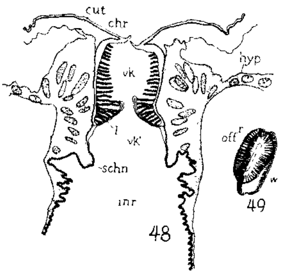


Abb. 48. Horizontaler Schnitt durch ein Stigma.

Abb. 49. Flächenansicht des Stigmas.

chr Chitinring, cut Cuticula, hyp Hypodermis, inr Innenraum des Tracheenbüschels, l Lippe, öff Trichteröffnung, r Trichterrand, schn Einschnürung zwischen der Vorkammer und dem Tracheenbüschelinnenraum, vk äusserer Abschnitt der Vorkammer, vk′ innerer Abschnitt derselben, w Wand des äusseren Abschnittes der Vorkammer.

Der Trichter könnte wohl mit dem Krancherschen Lippengebilde identifiziert werden. Er ist also dem Haarfilter der Seidenraupe gleichzusetzen. Innerhalb der Lippe kommt ein zweiter Hohlraum als direkte Fortsetzung des ersten vor, an welchen durch eine Art Einschnürung noch ein dritter angrenzt. Der erste und zweite Raum machen zusammen die Vorkammer aus, während der dritte die Stelle darstellt, wo der Verbindungskanal mit dem Längsstamme zusammentrifft. Von derselben Stelle entspringt auch der Büschel der Tracheen, welche sich auf die ganzen Organe des Segmentes verteilen. Die Vorkammer ist mit einem sehr

hohen Epithelium ausgekleidet, dessen Zellen einen langen, zu der Oberfläche senkrecht gelegenen Kern haben. An dem Chitinüberzug der Wand der Vorkammer fehlen die Spiralfalten. Ein Verschlussapparat ist nicht ausgebildet. Sonst soll er an der Einschnürung zwischen der Vorkammer und dem Büschelinnenraume nachzuweisen sein. Das Lippengebilde mag dazu dienen, die mit der Luft hineinkommenden Fremdkörper von dem Eindringen in die Tracheen zu verhindern.

Das Tracheenepithel (Abb. 50) besteht aus platten, ziemlich umfangreichen, polygonalen Zellen mit einem rundlichen Kern. Der letztere ist von feineren Körnchen ausgefüllt. Die Epithelzellen liegen aussen einer dünnen Basalmembran auf. Innen sind sie in die Intima differentiiert, welche spiralig gefaltet ist. Wie allgemein bekannt, wird durch die engen Umgänge der Spiralfalte das Tracheenrohr vom Zusammenfallen bewahrt. Nur in den Endfäden, den Tracheenkapillaren, fehlt die Falte vollkommen. Wo das Tracheenrohr in die Kapillare übergeht, dort werden stets ein oder mehrere, selbständige, grosse Wandzellen, die sogenannten Übergangszellen (Abb. 51), aufgefunden, in welchen das bis dahin interzelluläre Lumen zu einem intrazellulären wird. —

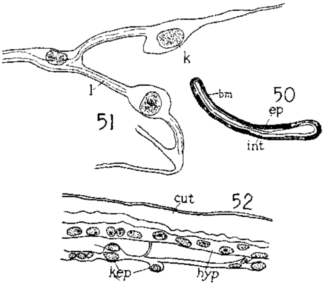


Abb. 50. Quer durch den Tracheenlängsstamm.
Abb. 51. Übergangszellen.
Abb. 52. Tracheenendigungen an der Hypodermis, ein transversaler Schnitt durch die Innenfläche eines Abdominalfusses, Dörnchenfortsätze ausgelassen.
bm Basalmembran, cut Cuticula, ep Tracheenepithel, hpy Hypodermis, int Intima, k Kern der Übergangszelle, kep Kerne der Kapillarenepithelzellen, l Lumen der Trachea.

Die Kapillaren (Abb. 52) scheinen wesentlich aus Chitin gebildet zu sein. Sie sind überall mit zahlreichen, kleineren Zellen, Kapillarenepithelzellen, besetzt, die sich wie die Übergangszellen in der Regel von der Wand des Röhrchens deutlich abheben. Das Lumen lässt sich in den Kapillaren nicht scharf beweisen. Ich habe die Tracheenendigungen an der Hypodermis betrachtet. Dort scheinen die einzelnen Kapillarenenden von unten in die Zellenschicht hineinzudringen. Die eigentliche Atmung findet wahrscheinlich in den Kapillaren statt.

X. Zirkulationsorgane und Leibeshöhle.

a. Das Herz.

Der Herzschlauch verläuft median dicht unter der Rückenhaut (Abb. 55). Unten kommt er mit dem dorsalen Diaphragma fast in Berührung, ohne doch mit demselben wirklich verwachsen zu sein. An der Rückenhaut sowie auch an dem Diaphragma ist das Herz mittels zahlreicher, feinster, bindegewebiger Fädchen (Aufhängefäden) aufgehängt.

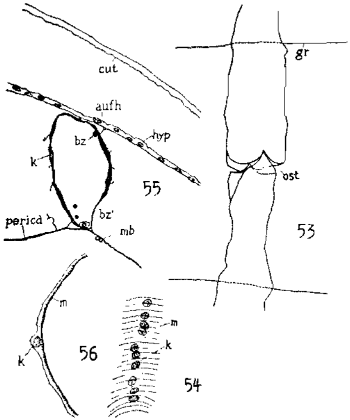


Abb. 53. Herzschlauch, von unten gesehen.
Abb. 54. Flächenansicht der Herzwand, von der Seite gesehen.
Abb. 55. Quer durch den Herzschlauch zwischen den Ringmuskeln.
Abb. 56. Quer durch die laterale Wand des Herzens.
auf Aufhängefaden, bz gewöhnliche Blutzelle, bz′ grosse, elliptische Blutzelle, cut Cuticula, gr Segmentgrenze, hyp Hypodermis, k Kern der Herzwand, m Ringmuskel, mb Medianbündel der Fettzellenaufhängsel, ost Ostium, pericd Pericardialdiaphragma.

Das Lumen ist nie gekammert, wie es von manchen Autoren behauptet worden ist, sondern besteht nur aus einem einzigen langen Hohlraum eines Schlauches, welcher zwar in der Mitte jedes Segmentes mit je einem Paar nach hinten gerichteten Ostien versehen ist, die dem Herzen das Aussehen verleihen, als ob es in mehrere Kammern geteilt sei (Abb. 53).

Das Lumen ist von einer äusserst dünnen Wand umgeben (Abb. 56), die quergestreifte Ringmuskeln enthält, welche in gleichmässigen Abständen regelmässig auf derselben angereiht sind. Die Aussenfläche der Wand ist mit einer dünnen Schicht von Sarcoplasma überzogen, worin auf jeder Seite ziemlich grosse, abgeplattete, rundliche Kerne in eine Längsreihe angeordnet vorkommen. Wo die Kerne sich befinden, dort schwillt die Plasmaschicht etwas nach aussen an.

Es ist sehr bemerkenswert, dass jeder Ringmuskel von je zwei Kernen begleitet ist (Abb. 54). Diese Tatsache deutet ohne Zweifel auf die Zweizelligkeit des Muskelringes hin. Das Paar Kernreihen erinnert uns sehr an die Entwicklungsweise des Herzschlauches aus zwei seitlich gelegenen, embryonalen Gewebestrifen, den Cardioblasten. In dem Herzlumen sind sowohl gewöhnliche, als auch grosse elliptische Blutzellen (siehe unten) anzutreffen (Abb. 55).

b. Diaphragmata.

Der Körperhohlraum ist von Diaphragmaten in einige Abschnitte geteilt (Abb. 57). Sie sind eine sehr zarte, durchsichtige, mehr oder minder lückenhafte, strukturlose Membran. Die Leibeshöhlenabschnitte werden von ihnen nicht vollständig von einander abgeschlossen, sondern durch sie kann sich das Blut der einzelnen Räume unter einander vermischen. Es sind in der Blattwespenlarve fünf Bluträume vorhanden, d. h., ein dorsaler Pericardial-, ein ventraler Perineural-, ein Paar laterale, noch nicht beschriebene, und endlich ein in der Mitte liegender Circumintestinalsinus. Der letztgenannte umschliesst den Darmkanal. Der Pericardialsinus enthält das Herz und wird von dem darunterliegenden Darmsinus durch das Pericardialseptum gesondert, wähend das Hyperneuraldiaphragma von dem circumintestinalen den Nervensinus trennt, den die Nervenkette durchzieht. Bei allen bisher betrachteten Insekten fehlen die Septen zwischen dem Darm- und dem Lateralsinus, so dass der Darmsinus der übrigen Insekten auch den Lateralsinus mit in sich einschliesst. Die betreffenden Sep-

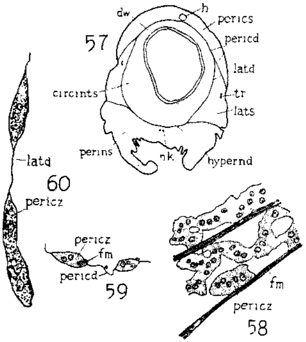


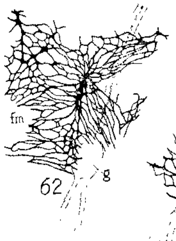
Abb. 57. Quer durch den Hinterleib, die Verteilung der Bluträume veranschaulichend.
Abb. 58. Flächenansicht des Pericardialdiaphragmas.
Abb. 59. Sagittalschnitt durch dasselbe.
Abb. 60. Quer durch das Lateraldiaphragma.
circints Circumintestinalsinus, dw Darmwand, fm Fächermuskel, h Herz, hypernd Hyperneuraldiaphragma, latd Lateraldiaphragma, lats Lateralsinus, nk Konnektiv der Nervenkette, pericd Pericardialdiaphragma, perics Pericardialsinus, pericz Pericardialzelle, perins Perineuralsinus, tr Tracheenlängsstamm.

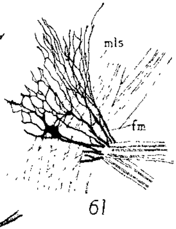
ten mögen Lateralsepten genannt werden. Den Lateralsinus durchzieht der Tracheenlängsstamm.

Alle Organe finden sich an die verschiedenen Bluträume verteilt. Der Circumintestinalsinus enthält ausser dem Darmtraktus noch die Spinndrüssen und die malpighischen Schläuche dazu, während die Muskeln der Körpermuskulatur hauptsächlich in den vier anderen Räumen aufgefunden werden. Die Fettzellen kommen in allen Räumen vor, wogegen die Oenocyten ausschliesslich in den peripheren zu liegen scheinen. Die Blutzellen sind freilich allenthalben anzutreffen.

Auf der dorsalen Oberfläche des Pericardial-, sowie auch der ventralen des Hyperneuraldiaphragmas liegen die sogenannten Fächermuskeln dicht an. Sie sind quergestreifte Muskelfasern, welche an den Stellen der Körperwand, wo das Pericardial-, beziehungsweise das Perineuralseptum mit den Segmentgrenzen zusammemtrifft, entspringen und sich divergierend auf die ganze Oberfläche des betreffenden Septums ausbreiten, wodurch eine fächerförmige Gestalt zustande kommt. Die Ansatzpunkte der Fächermuskeln sind zu gleicher Zeit dieselben der Muskelfasern der allgemeinen Körpermuskulatur, was auf den gemeinsamen Ursprung beider Muskelarten hindeuten könnte (Abb. 58–59, 61–62).

In der Mitte des Hyperneuraldiaphragmas anastomosieren die Fächermuskeln der beiden Seiten zu einem Retikulum (Abb. 62), das in der Segmentmitte, d. h., oberhalb des Ganglions besonders kräftig





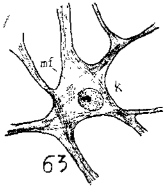


Abb. 61. Fächermuskel des Hyperneuraldiaphragmas, von oben gesehen.

Abb. 62. Muskelretikulum desselben, von oben gesehen.

Abb. 63. Eine Zelle des Retikulums.

fm Fächermuskelfaser, g Ganglion, k Kern der Retikulumzelle, mf Muskelfibrille, mls Musculus longitudinalis sterni.

entwickelt ist. An den Stellen, wo die Fäden des Retikulums miteinander zusammenstossen, ist ein Plasmahaufe vorhanden, worin ein grosser, rund-

licher Kern eingebettet liegt, um welchen herum die Muskelfibrillen einzelner Fäden mit einander gekreuzt laufen, damit dieselben eines jeden Fadens an die anderen verteilt werden (Abb. 63). Dem Aussehen nach könnte die Zelle wohl mit gewissen Nervenzellen der Wirbeltiere, den sogenannten multipolaren Ganglienzellen verwechselt werden.

Von den Fächermuskeln des Pericardialdiaphragmas treten dieselben der einen Seite keineswegs mit denen der anderen Seite in Verbindung, sondern gehen unterhalb des Herzens, jeder für sich, zwar in feinere Endfäden geteilt, frei auf dem Septum aus (Abb. 64). Sie stehen mit dem Herzschlauch in keinerlei Beziehung, sodass sie mit der Diastole des Herzens nichts zu tun haben. Das Pulsieren des letzteren scheint ausschliesslich von den Ringmuskeln der Schlauchwand bewirkt zu werden.

Das Pericardialdiaphragma ist im Zustande der Ruhe dorsalwärts vorgewölbt, im Kontraktionszustande der Fächermuskeln horizontal gespannt. Die Spannung erweitert den Pericardialsinus, und veranlasst den Eintritt des Blutes aus dem Darm- und den Lateralsinus in den Pericardialraum, aus welchem es in das Herz fliesst. Das Hyperneuralseptum hingegen ist im Ruhezustand nach unten gebogen, sodass der Druck der Muskelspannung das Blut in den Neuralraum hineinführt, während die Entspannung es aus demselben in den Darm- und den Lateralsinus presst. Durch Zusammenwirken der beiden Septen könnte also ein ziemlich gleichmässiger Kreislauf der Hämolymphe herbeigeführt werden (Abb. 57).

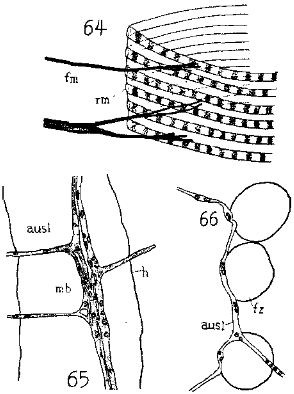


Abb. 64. Fächermuskelendigungen auf dem Pericardialseptum.
Abb. 65. Medianbündel der Fettzellenaufhängsel, von unten gesehen.
Abb. 66. Seitenausläufer desselben, an die Fettzellen herantretend.
ausl Ausläufer, fm Fächermuskelfaser, fz Fettzelle, h Herz, mb Medianbündel, r Ringmuskel der Herzwand.

Aus der ventralen Oberfläche des Pericardialseptums läuft median ein Bündel bindegewebiger Fäden, von welchem, in fast regelmässigen Abständen, Seitenfäden ausgehen, die, nach latero-ventralwärts verlaufend, in

Endverzweigungen übergehen, an denen die Fettzellen des Darmsinus hängen (Abb. 65–66). Da in den genannten Fäden die Fibrillen völlig fehlen, so könnten sie vielleicht als Glattmuskeln gedeutet werden. Abgesehen davon sind aber bei der Blattwespenlarve die Muskeln sowohl der Körper- als auch der Visceralmuskulatur sämtlich quergestreift und mit Kernen versehen. *Die Fettzellen des Pericardialsinus sind mit feinen Fäden einzeln an die Herzwand angeknüpft.* Sie sind, abgesehen von denen des Darmsinus, besonders reichlich in dem genannten Raum, und zwar zu beiden Seiten des Herzens angehäuft.

In den Septen liegen ferner zahlreiche Pericardialzellen (Abb. 58–60). Sie machen zusammen ein lockeres, einschichtiges Gewebe, das in Flächenansicht einen wolkenartigen, unregelmässigen Umriss bietet, dessen Randpartien, wie es sich in einem Querschnitt klar verrät, allmählich in die reine Membran des Septums ausgehen. Die Zellen liegen trotzdem in den meisten Fällen nicht dem Diaphragma auf, sondern sind in demselben derart eingelagert, dass sie auf den beiden Flächen von einer dünnen Lamelle überzogen sind, die sich, wo die Zellen fehlen, direkt in die Membran des Diaphragmas fortsetzt. Die Pericardialzellen scheinen also vorzüglich die Bildungszellen der Septen zu sein. Man trifft sie in der Tat auf jedem Diaphragma der Wespenlarve, obwohl sie in den dorsalen Teilen des ganzen Septensystems weit dichter verteilt sind als in den ventralen Gegenden.

c. Die Blutzellen.

An den Blutzellen sind folgende zwei Sorten zu unterscheiden: 1. Gewöhnliche Leucocyten mit einem chromatinärmerern, jugendlichen Kern. Das Zytoplasma ist homogen. Es ist, im Verhältnis zum Kern, bald reichlich, bald spärlich vorhanden. Der Kern ist mit wenigen verschieden grossen, unregelmässig verteilten Chromatinkörnchen versehen. Die Form und die Grösse der Zellen sind nicht bestimmt (Abb. 67.) 2. Grosse elliptische Zellen mit einem kreisrunden Kern, der gleichmässig von Chromatinkörnchen ähnlicher Grösse ausgefüllt ist. In ihm sind, wie es in den gewöhnlichen Zellen auch der Fall zu sein scheint, keine Nukleolen zu bemerken. Die Zelle ist mit reichlichem, homogenem Zytoplasma versehen. Ihre Form und Grösse scheinen fast bestimmt zu sein (Abb. 68).

Die gewöhnlichen Leucocyten kommen in allen Gegenden des Körperhohlraums vor, und zwar entweder vereinzelt oder auch zu Haufen ange-

sammelt (Abb. 69). Im letzteren Falle könnten sie, wie schon beschrieben, mit den Muskelbildungszellen verwechselt werden. Die Haufen werden meistens in den peripheren Bluträumen aufgefunden.

Die elliptischen Zellen sind, den gewöhnlichen Leucocyten gegenüber, *nur selten anzutreffen. Sie könnten wohl von den letzteren abgeleitet* werden.

XI. Fettzellen.

Die Fettkörper kommen in Form einzelner Zellen (Abb. 70) überall in der ganzen Leibeshöhle vor. Der Kern ist mit zahlreichen Chromatingranülen und einigen Nukleolen ausgerüstet. Er ist von den das Zytoplasma ausfüllenden, grossen Fettkugeln stark zusammengedrückt. Die Fettzellen nehmen eine rundliche Gestalt an, die in der Mitte mit einem Kern versehen ist. Der Zellkörper ist ausserordentlich gross. Die Nukleolen des Kernes sind im Zerfallen begriffen, sodass in älteren Zellen davon keine Spur mehr übrig bleibt.

Ausser gewöhnlichen Fettzellen kommen auch die Henneguyschen Calcospheriten (Abb. 71–72) reichlich vor. Sie sind viel kleiner als die Fettzellen, aber in fast unzählbarer Anzahl vorhanden. Es dürfte sich hier vielleicht um eine Harnzelle handeln, deren Zellkörper vollkommen von einem doppelt brechenden, rundlichen Kristalle ausgefüllt ist, welches mutmasslich aus irgendeiner Art Harnsäuresalz besteht. In ihr habe *ich die von* Henneguy bemerkte, konzentrische Schichtung nicht beweisen können. Der Kern und das Plasma scheinen völlig verschwunden zu sein.

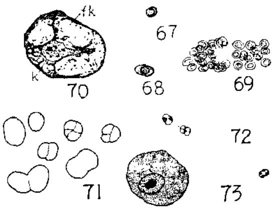


Abb. 67. *Gewöhnliche Blutzelle (Proleucocyte).*
Abb. 68. Grosse, elliptische Leucocyte.
Abb. 69. *Haufen der Proleucocyten,* Punktierung des Plasmateils ausgelassen.
Abb. 70. Fettzelle.
Abb. 71. „Calcosphérites".
Abb. 72. Dieselben, durch gekreuzte Nicolprismen gesehen.
Abb. 73. Oenocyte.
fk Fettkugelvakuole, k Kern der Fettzelle.

Die Calcospheriten bestehen gelegentlich aus zwei, drei oder vier mit einander verwachsenen Kristallen, wodurch ein zusammengesetztes, zwei-, drei- oder vierlappiges Gebilde zustande kommt.

XII. Oenocyten.

Die Oenocyten (Abb. 73) sind diffus in dem Körperhohlraum verteilt. Sie kommen meistens vereinzelt zwischen den Fettzellen vor, und liegen wie die letzteren mit feinen, bindegewebigen Fäden fixiert in der Leibeshöhle. In Grösse und Form könnten sie mit den Fettzellen verglichen werden; nur ist ihr Vorkommen weit seltener als das der letzteren. Der rundliche, chromatinreiche Kern, welcher stets in der Einzahl vorhanden ist, hat einen ziemlich grossen, scharf umgrenzten Nukleolus in der Mitte, während das wohl entwickelte Zytoplasma auffallend homogen und hell erscheint. Die Oenocyten sind also in gewisser Hinsicht den Blutzellen ähnlich, von welchen sie aber in Grösse auf den ersten Blick unterschieden werden können. Sie scheinen ektodermalen Ursprungs zu sein. Ihre Funktion ist noch nicht sicher bekannt.

Biologische Betrachtungen.

I. Vorbemerkung.

Für biologische Betrachtungen habe ich in dem hiesigen Laboratorium Züchtungsversuche angestellt, wozu mehrere, mit feinsten Drahtnetzen bedeckte Zuchtkasten zurechtgemacht wurden. In jedem Kasten befand sich ein Topf, worin ein Kiefersprössling gepflanzt war. Das Pflänzchen wurde von Zeit zu Zeit gewässert. Bei heiterem Wetter habe ich die Kasten ins Freie gebracht, um die Pflänzchen das direkte Sonnenlicht geniessen zu lassen. Sonst standen sie auf einem Tisch nahe an den südlichen Fenstern des Laboratoriums. Die Topferde war mit abgefallenen, dürren Kiefer-Blättern bedeckt.

Die überwinternden Kokons hatte ich nun in der Pflanzschule gesammelt und in den Kasten eingesetzt, indem sie auf der Blattdecke der Topferde an das Stämmchen des Sprösslings angelegt wurden. Im freien sind die Kokons entweder zwischen abgefallenen Blättern auf dem Boden, oder auch auf der Stammrinde nahe demselben aufzusuchen. Sie nehmen eine elliptische Form von etwa 7 mm. Länge und etwa 4 mm. Breite an. Sie sind aschbraun mit schwachem Seidenglanz und lassen sich leicht querreissen.

II. Betrachtungen an den Imagos.

Die Larven schlüpften vom Ende April bis zum Ende Mai fortwährend aus dem Kokon heraus, und zwar schufen sie dazu an einem Ende

des Kokons derart ein Schlupfloch, dass mit den Mundwerkzeugen an der genannten Stelle ein kreisförmiger Abschnitt aus der Wand ausgeschnitten wurde, der aber mit dem übrigen Teil des Kokons an einem Punkte noch in Verbindung stand, wo die Wandschichten vom Kauen verschont blieben, sodass ein kleines, uhrschalenartiges Deckelchen *dadurch zustande gekommen* war (Abb. 74). Der Deckel wurde nun von innen abgehoben, und die Wespe kam durch das so entstandene Loch aus dem Kokon heraus. Bei den soeben ausgeschlüpften Imagos war das Flugvermögen noch kaum entwickelt. Sie schüttelten unaufhörlich die beiden Antennen. Häufig wurden sie auf der Blattdecke des Topfes das für das Pflänzchen gegossene Wasser aufleckend gefunden. (Wie allgemein bekannt, überwintern die Seidenraupen in Eiern. Die Imagos treten aus dem Kokon dadurch heraus, dass der letztere an einem Ende durch Mundsaft aufgeweicht wird.)

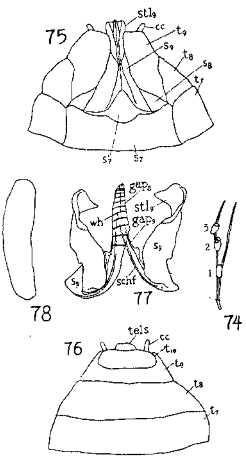


Abb. 74. Sommerkokons auf einem lebenden Blatt.

Abb. 75. Unterseite des Körperendes des weiblichen Imagos.

Abb. 76. Rückenseite desselben.

Abb. 77. *Die ganzen Sägewerkzeuge* mit der Scheide herauspräpariert, von unten gesehen. (Die linke Gonapophyse und die linke Hälfte des Sternites des achten Segmentes sind abgenommen).

Abb. 78. *Das Ei.*

1 puppenhaltiger Kokon, 2 Kokons nach dem Austreten des Imagos, 3 derselbe, dem der Deckel des Schlupflochs abgefallen ist, cc Cerci, gap Gonapophysen, s Sternite, s′ der hintere Teil des 7. Sternites, ein häutiges Läppchen, das die Basis der Scheidenspalte überdeckt, schf Schienenführung, stl Styli, t Tergite, oder noch zutrefflicher Tergite plus Pleurite, tel Telson, oder Afterklappen (Analsegment), wh Widerhaken.

Die Paarung dauerte längere Zeit. Die gepaarten Tiere liefen in dem *gekoppelten Zustand lebhaft herum.* Die Eiablage geschah an dem Mittelpunkt eines lebenden Blattes, und zwar erst nach einem gründlichen Ausprüfen desselben, indem das Tier ein- oder zweimal auf dem Blatt die ganze Strecke hin und zurück kroch. Dann setzte es sich auf das Blatt fest, streckte die Hinterbeine aus, und schmiegte das Körperende dicht an die Blattoberfläche an, um auf derselben mit dem Sägeapparate einen Längsschlitz herauszuarbeiten, wobei die beiden Hälften der Sägescheide fortdauernd wechselseitig hin und zurückgezogen wurden.

Der Sägeapparat (Abb. 75–77) besteht aus einem Paar Sägeborsten (Sägestacheln), den umgewandelten Gonapophysen des achten Segmentes, sowie auch aus einer Stachelrinne der verwachsenen Gonapophysen des neunten Segmentes. Die Rinne ist nach unten zugekehrt. An den freien Kanten ihrer Seitenwände verläuft je eine Chitinleiste, die in eine Furche der Stachelinnenfläche genau passt, sodass die Bewegung der Sägeborsten zwangläufig erfolgt. Die letzteren sind auf der Aussenfläche mit mehreren Querreihen von Widerhaken ausgerüstet. Die Sternite des achten und neunten, und die Styli des neunten Segmentes machen zusammen die Sägescheide aus, worin eingelegt sich der Sägeapparat befindet.

Tabelle II. Schematische Übersicht der Abdomengliederung des weiblichen Imagos.
× bedeutet *Lage (proximal) der Gonapophysen.*
○ „ dieselbe (distal) der Styli,
◎ „ dieselbe (wahrscheinlich zwischen der Tergiten- und der Pleuritenreihe hinter dem zehnten Segment) der Cerci,
} „ Verwachsen der betreffenden Sklerite.

Tergite	1 }	2 }	3 }	4 }	5 }	6 }	7 }	8 }	9 }	10 }	◎
Pleurite	1 }	2 }	3 }	4 }	5 }	6 }	7 }	8 }	9 }	10 }	→ After
								×	× ○		
Sternite		2	3	4	5	6	7	8	9		
								↓ Vagina			

Nun schien es als ob der Schlitz auf dem Blatt durch die Sägeborsten noch erweitert würde. In die soentstandene Rinne wurden dann die Eier eingekittet. Die Länge der Rinne betrug ca. 2 mm Ihre Tiefe erreichte fast die andere Seite der Blattnadel. Das längliche, etwas gebogene, stark abgeplattete Ei (Abb. 78) ist etwa 1,5 mm lang. Im Vergleich mit dem Körper ist es also auffallend gross. Die Eiablage dauerte etwa anderthalb Minuten, wonach das Tier ohne Rücksicht von dem Ort abflog. Zehn Minuten später aber wurde die zweite Abgabe von demselben Tier und zwar auf einem anderen Blatt angefangen. Das Ablegen ging also mehrmals vor sich, und von einem Tier wurden dabei mehrere Blätter angegriffen. Die Ablage konnte ich auch am nächsten Tage an demselben Tier beobachten; vielleicht dauerte sie noch mehrere Tage fort. Bei der Eiablage schienen jüngere Blätter vermieden zu werden. Nach zehn Tagen schlüpften junge Larven schon aus der Rinne der Blattoberfläche heraus, wo ein in der Mitte längsgerissenes, weisses Häutchen hinterlassen war, das aus dem nun weit geöffneten Spalte hervorragte.

III. Betrachtungen an den Larven.

Das Ausschlüpfen der Larven geschah von Mitte Mai bis Anfang Juni. Bei der Nahrungsaufnahme sitzen sie mit dem nach der Blattspitze gerichteten Kopfe derart auf einer Seite des Blattes, dass das letztere zwischen den beiden Reihen der Abdominalfüsschen festgehalten wird. Es kann dabei dem letzten Paar Füsschen allein zukommen, das ganze Körpergewicht auf die Unterlage zu stützen. Zum Festhalten der Blattnadel mögen vielleicht der Schleim aus den Hautwarzen viel beitragen, mit welchem der Körper oder die Füsschen auf das Blatt aufgeklebt werden. Der Frass beginnt an der Spitze und schreitet nach der Basis zu fort. Die jüngeren Larven sitzen je zwei oder drei auf einem Blatt, und fressen es gemeinsam von beiden oder allen Seiten her, wobei der härtere Teil in der Achse fadenartig übriggelassen wird. Von einer erwachsenen Larve wird je ein Blatt mit Haut und Haar verzehrt. Nur das proximale Ende desselben bleibt vom Frass verschont. Jeder Bissem wird von einem Kopfabheben begleitet, sodass das Frassbild von demselben der Seidenraupen ziemlich verschieden ist, bei welch letzteren der Kopf eine Zeit lang an die Blattkante angeschmiegt bleibt.

Am Ende Juni habe ich die Vorbereitung zum Einsperren beobachten können. Wenn die vorletzte Häutung herannaht, (die letzte Häutung geschieht im Kokon im Frühling des nächsten Jahres) wird die Nahrungsaufnahme eingestellt, die grünliche Färbung geht allmählich aus, und der Körper scheint etwas abgekürzt zu werden. Schliesslich setzt sich die Larve zur Häutung an ein Blatt fest. In dieser Zeit ist sie auf der Rückenseite rotbraun, auf der Bauchseite gelblich geworden, und zwar zeigen die Tergiten der Brustregion nun wieder eine leicht grünliche Tinktur. Als Unterlage der Häutung scheinen verdorrende Blätter am Zweige bevorzugt zu werden.

Wenn das Tier aus dem alten Häutchen herauskroch, war ihm der Genalfleck fast ausgegangen, und zwar abgesehen davon, dass ein leichter Abdruck zurückgeblieben war, worin nun das dunkle Auge auffallend hervortrat. Die schwarze Färbung der Brustbeinglieder war vollkommen verschwunden. Die Körperlänge betrug nur 1,7 cm, während sie vor der Häutung fast 2 cm erreichte.

Die Beobachtung der Häutung wurde am Nachmittag gemacht. Am andern Morgen befand sich das Tier schon im Kokon eingesperrt. Also scheint dazwischen keine Nahrungsaufnahme eingeschaltet gewesen zu sein.

Die Kokons werden mit Vorliebe zwischen den lebenden Blättern oder

an deren Basis gebaut. Häufig sind sie aber auch an den Zweiglein aufzufinden. Am Kopfende des Kokons wird dann das Schlupfloch durchgebohrt, wodurch der Vollkerf nun herauszukriechen hat.

Das Ausschlüpfen der Imagos wurde seit Mitte Juli bemerkt, die Larven aus den Eiern derselben schlossen sich schon Anfang Oktober in den Kokon ein. Sie überwintern in diesem Zustand, um sich darin erst im nächsten Frühjahr zu verpuppen. Die Blattwespen kommen also jährlich zweimal hervor.

Bemerkungen über die Abwehr.

I. Modus der Beschädigung.

Es handelt sich hier um einen von den kauenden Mundwerkzeugen der Larven hervorgebrachten Frassschaden. Alle Kieferarten können von *Nesodipron japonica* befallen werden. Besonders sind aber die Beschädigungen an *Pinus densiflora* Sieb. et Zucc., sodann auch an *Pinus thumbergii*, Parl. auffällig. Neben *Dendrolimus remota* Walk., stellen sich in Japan *Nesodipron japonica* als die ärgsten Schädlinge der Kieferwälder dar. Kränkelnde Bäume werden häufiger angegriffen als gesunde. Junge Bäume und besonders Sprösslinge scheinen von den Larven bevorzugt zu werden, wo sie stets in enormen Gruppen auf den Blättern anzutreffen sind. Wie bemerkt, werden häufig die befallenen Pflanzen der grünen Krone völlig beraubt. Junge oder schwächliche Bäume können gelegentlich sogar zugrunde gehen.

Die Larven der ersten Generation nähren sich in der Regel von den Blättern des vergangenen Jahres, während dieselben des zweiten Auftretens mit Vorliebe die neuen Blätter angreifen, ohne dass jedoch eine strenger Wahl der Nahrung dabei zu herrschen scheint.

II. Vorbeugungsmassnahmen.

1. Man muss immer für die stärkere Entwicklung der Bäume sorgen.
2. Schwächliche Bäume sollen möglichst schnell abgehauen und verwertet werden.
3. Waldvögel dürften beschützt werden, da sie mit Vorliebe die Larven suchen.
4. Auch Mäuse, Erdmäuse und Eichhörnchen, welche während des Winters die Larven im Kokon fressen, bedürfen des Schutzes.

5. Endlich gibt es Fliegen und Schlupfwespen, die in den Larven und Puppen schmarotzen. Sie könnten mit Vorteil in Schutz genommen werden. Während der beschriebenen Züchtungsversuche kamen sowohl eine Art Schlupfwespen als auch eine Schmarotzerfliege in den Kasten vor.

III. Mittel zur Vertilgung.

Wenn man einmal die Schädlinge auf einem Baum oder in einem Waldteil entdeckt, so muss der angegriffene Baum oder der befallene Ort sofort mit einem offenen Graben umzogen, oder auch die Bäume der sich ausstreckenden Äste beraubt werden, um die Verbreitung der Schädlinge möglichst einzuschränken. Erst dann können die verschiedenen Vertilgungsmassnahmen erfolgreich ausgeführt werden. Die letzteren lassen sich am bequemsten an den Larven und Puppen anwenden.

Die sich auf den Sprossen ansammelnden Larven können samt denselben abgepflückt, oder auch an Ort und Stelle mit den blossen Händen zerquetscht werden. Im letzteren Falle werden grobe Handschuhe zu dem Vernichten viel beitragen und zu gleicher Zeit die Hände schützen. Wenn die Schädlinge an hohen Bäumen aufgetreten sind, müssen sie früh am Morgen, wenn es noch kühl ist, vom Stamm abgeschüttelt werden. Dieselben an den jüngsten Pflänzchen kann man auch in eine mitgebrachte Blechbüchse abbürsten, welche Wasser mit Petroleum enthält.

Wenn es sich um eine Pflanzschule oder ein kleines Waldstück handelt, so können bequem verschiedene Spritzmittel angewandt werden. Als solche kommen in erster Linie Petroleumemulsionen und Seifenlösungen mit oder ohne Pyrethrum-extrakte in Betracht. Auch blosses Bespritzen mit starken Wasserstrahlen könnte die Larven vernichten.

Die Kokons der ersten Generation lassen sich leicht aus den Zweigen sammeln, um sofort getötet zu werden.

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Nematodes in the Paddy Field, with Notes on their Population before and after Irrigation.

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With forty-eight Text-figures.

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Introductory.

Living free in the soil and fresh water are found various kinds of nematodes, of which some infest various plants and animals in the course of their life while the others pass a free life throughout the cycle. Belonging to the former are the Ancylostomidae, Strongylidae, Mermithidae, some species of the Anguillulinidae and Rhabditidae, etc. Among the latter the Anguillulinidae and Rhabditidae are the most prevalent. It is therefore of the utmost agricultural importance to reveal the species of soil nemas, their bionomics and other facts. With regard to these problems investigation was undertaken for the past seventy years by many authorities, such as BASTIAN, BÜTSCHLI, DE MAN, COBB, MICOLETZKY, MAUPAS, MARCINOWSKI, v. DADAY, JÄGERSKIÖLD, STEINER, MENZEL,

BRAKENHOFF, DITLEVSEN, HOFMÄNNER, STEFANSKI, et al. In spite of their microscopic size, the nemas present a wonderful complexity in organization and an astonishing adaptability and resistance to environmental complexes, such as the chemical and physical changes of the soil and fresh water, food and other factors. The food habits, though highly difficult, are classified according to various types. Some are parasitic or semiparasitic of the higher plants and take mainly vegetable juice for their nourishment, for example, *Heterodera*, *Tylenchus*, *Aphelenchus*, *Dorylaimus*, etc. Some are carnivorous and devour various kinds of microzoa (Protozoa, Rotatoria, Oligochaeta, Tardigrada, and some injurious nemas), for example, *Mononchus*, *Tripyla*, *Trilobus*, *Ironus*, etc. Some are saprophitic, being usually found in decaying organic matter, for example, *Cephalobus*, *Rhabditis*, *Diplogaster*, *Bunonema*, etc. Some are polyphagus and feed upon living microorganisms and decaying plant and animal matter in the water. The majority of the fresh water nemas belong to this group.

It has hitherto been revealed by the soil census that the free-living nemas are inconceivably abundant so that they probably constitute one of the most important mechanical and biological factors in the soil as well as at the bottom of the lake and river. Among them, the forms which are armed with the oral spear amount to a large number and are generally thought to be injurious to the roots of the higher plants and the crops; accordingly much attention has recently been paid to their economic importance. In spite of such urgent need no systematic research has up to the present been attempted in Japan. Under these circumstances, the present work was undertaken mainly for the classification of the nematodes in the paddy field of the University farm at Komaba near Tokyo, making an attempt to glance at their population before and after irrigation.

Here I wish to fulfil the pleasant duty of returning my hearty thanks to Professor T. KABURAKI for invaluable advice and courtesies shown me throughout the course of the present work and also to Mr. B. SHINDÔ for many courteous acts

Material and Methods.

The paddy field of the University farm subjected to examination is isolated in a limited area and is generally irrigated from the middle of June to the beginning of November. The spring near the field is usually employed as the water source. After the harvesting of the rice plant nothing is cultivated in the field.

In the experiment the superficial soil mass, 10 inches in depth, was subjected to examination and it was taken midway between two rows of stumps or plants by means of a soil sampling tube, which corresponds in cross section to about one millionth of an acre. It was taken up in five layers, namely I from the surface to the depth of 2″, II from 2″ to 4″, III from 4″ to 6″, IV from 6″ to 8″ and V from 8″ to 10″. On account of the muddy character of soil especially in the irrigation season, it is quite difficult to remove the samples in each layer in exact order. For this reason, a special ten-inch tube made up of five 2-inch tubes inserted in it was always used.

The first plot was taken at the beginning of June before irrigation. Ploughing and manuring were soon carried out until the young rice plants were transplanted from the seedling bed. Then the field was irrigated. The second plot was taken at the middle of July, about a month after irrigation.

In order to obtain reliable data 4 soil samples taken from the same layer were mixed together and then subdivided into aliquot parts.

For separating the nemas from the soil, various sieves were employed in order, the number of the meshes per inch being 16, 20, 40, 80, 120, 160, and 200. Sometimes the soil was mixed up with water and then filtered through these sieves and repeatedly washed by a stream of water.

For careful identification and detailed study of morphology the nematodes are placed in a drop of water at the middle of the slide glass and, after killing them by BUNSEN's flame, are covered with a cover glass.

For fixation and temporary preservation an alcohol-glycerin mixture (9 parts of 70% spirit and 1 part of glycerin) was always used.

Systematic Part.

Small as the examined area is, it furnishes us with an unexpectedly large number of the species. The number of individuals which came under my observation amount in all to 3,124, which are referable to 25 genera and 48 forms, including a new genus, 10 new species and 3 new varieties. The following is a list of the species as arranged according to BAYLIS and DAUBNEY's synopsis of the families and genera of nematodes.

List of the species dealt with in the present account.

Family Alaimidae MICOLETZKY.

1. *Alaimus primitivus* DE MAN.
2. *A. lemani* STEFANSKI.

3. *Bastiana exilis* COBB.
4. *Tripyla papillata* BÜTSCHLI.
Family Trilobidae MICOLETZKY.
5. *Cyatholaimus ruricola* DE MAN.
6. *Monhystera filiformis* BASTIAN.
7. *M. paludicola* DE MAN.
8. *M. agilis* DE MAN.
9. *Prismatolaimus dolichurus* DE MAN.
10. *Trilobus gracilis* BASTIAN.
Family Rhabditidae MICOLETZKY.
11. *Cephalobus elongatus* DE MAN.
12. *C. emarginatus* DE MAN.
13. *C. persegnis* BASTIAN.
14. *Chronogaster gracilis* COBB.
15. *Chriptonchus nudus* COBB.
16. *Plectus cirratus* BASTIAN.
17. *P. multitubiferus* sp. nov.
18. *Rhabdolaimus aquaticus* DE MAN.
19. *Walcherenia typica* DE MAN.
20. *Pseudoaulolaimus anchilocaudatus* gen. et sp. nov.
Family Oncholaimidae BAYLIS & DAUBNEY.
21. *Ironus ignavus* BASTIAN.
22. *Mononchus macrostoma* BASTIAN.
23. *M. tunbrigdensis* BASTIAN.
24. *Oncholaimus punctatus* COBB.
25. *Chromadola fluviatilis* COBB.
Family Anguillulinidae BAYLIS & DAUBNEY.
26. *Tylenchus gracilis* DE MAN.
27. *T. apapillatus* sp. nov.
28. *T. intermedius* DE MAN.
29. *T. filiformis* BÜTSCHLI.
30. *T. leptosoma* DE MAN.
31. *Aphelenchus bicaudatus* sp. nov.
32. *Criconema komabaensis* sp. nov.
33. *Tylencholaimus mirabilis* BÜTSCHLI.
34. *T. longicaudatus* sp. nov.
35. *T. bryophilus* sp. nov.
36. *Dorylaimus brigdammensis* DE MAN.
37. *D. carteri* BASTIAN.

38. *Dorylaimus elegans* var. *longicaudatus* var. nov.
39. *D.* *filiformis* var. *papillatus* var. nov.
40. *D.* *filiformis* var. *protumidus* var. nov.
41. *D.* *limnophilus* DE MAN.
42. *D.* *micoletzkyi* DE MAN.
43. *D.* *stagnalis* DUJARDIN.
44. *D.* *crassus* DE MAN.
45. *D.* *tambo* sp. nov.
46. *D.* *denticaudatus* sp. nov.
47. *Actinolaimus macrolaimus* DE MAN.
48. *Nygolaimus kaburakii* sp. nov.

I. FAM. Alaimidae MICOLETZKY, 1922.

1. *Alaimus primitivus* DE MAN, 1880.

Text-fig. 1.

No=179 ♀73 ♂30 Larvae 76 (Sex. coef.=41.1 n=103)
♀ L=1.21–1.65 mm. α=45.1–52.0 β=4.1–4.9 γ=12.6–17.1
V=31.6–48.0%
♂ L=1.39–1.49 mm. α=47.6–57.7 β=4.4–4.9 γ=12.9–17.4
Pn=4–5

The abbreviations used in the description of the species are as follows:—
No=Total individual number of a species.
L=Body-length.
α, β, γ, are the so-called DE MAN's proportional numbers, namely:

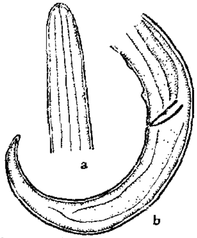
$$\alpha=\frac{\text{Body-length}}{\text{Width}}$$

$$\beta=\frac{\text{Body-length}}{\text{Oesophageal length}}$$

$$\beta'=\frac{\text{Body-length}}{\text{Length from head to median bulb.}}$$

$$\gamma=\frac{\text{Body-length}}{\text{Tail-length}}$$

V=Distance from the anterior end to vulva in percentage of the body-length.
Pn=Number of anal and preanal papillae.
Sex. coef.=Relative individual number of males against 100 females.
n=Total individual number of adults.
Rn=Number of cuticular rings.



Text-fig. 1. *Alaimus primitivus* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

Body very slender, tapering slightly forwards and extremely backwards. Cuticle unstriated, without bristle and lateral ala. Anterior end indistinct, without cephalic seta. Lip absent. Lateral organ invisible. Pharynx completely absent. Oesophagus elongate, gradually swollen behind. Female genital tube unpaired, postvulvar. Male genital tube single. Spicule short, fairly slender, without accessory piece. Caudal end of the male usually with a median row of 4–5 preanal papillae. Tail not so long, thread-like in both sexes.

2. *Alaimus lemani* STEFANSKI, 1914.

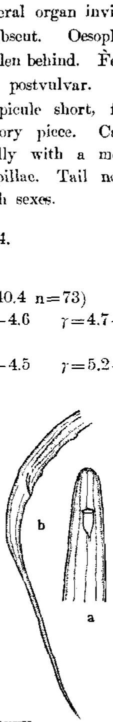
Text-fig. 2.

No=118 ♀52 ♂21 Larvae 45 (Sex. coef.=40.4 n=73)

♀ L=1.28–1.71 mm. α=42.2–56.3 β=4.1–4.6 γ=4.7–8.1
V=38.3–47.5%

♂ L=1.21–1.63 mm. α=47.1–61.8 β=4.0–4.5 γ=5.2–6.1
Pn=3

Body very slender, tapering towards both ends, especially behind. Cuticle unstriated, without bristle and lateral ala. Anterior end naked, without lip. Lateral organ large, handle-shaped. Pharynx completely absent. Oesophagus elongate, gradually swollen behind. Female genital tube single, postvulvar. Male genital tube single. Spicule very slender, without accessory piece. A median row of 3 preanal papillae is present in the male. Tail long, thread-like, ending in an extremely fine hair-like terminus in both sexes. In the female found in the paddy field the tail is much longer than that in the individuals dealt with by STEFANSKI.



Text-fig. 2. *Alaimus lemani* STEFANSKI.
a. Anterior part of the male.
b. Posterior part of the male.

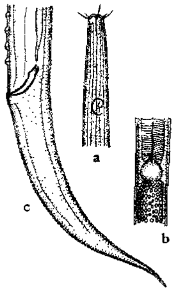
3. *Bastiana exilis* COBB, 1914.

Text-fig. 3.

No=14 ♀8 ♂1 Larvae 5 (Sex. coef.=12.5 n=9)

♀ L=1.42–1.83 mm. $a=60.0-65.4$ $\beta=4.9-5.3$ $\gamma=10.1-13.5$
V=57.3–60.1%

♂ L=1.27 mm. $a=70.6$ $\beta=3.9$ $\gamma=11.5$



Text-fig. 3. *Bastiana exilis* COBB.
a. Anterior part of the male.
b. Oesophagus-end region of the male.
c. Posterior part of the male.

Body extremely slender, tapering towards both ends. Cuticle finely striated, without bristle. Anterior end indistinct, without lip, but with 6 cephalic setae. Lateral organ spiral. Pharynx completely absent. Oesophagus elongate, gradually tapering towards the distal end, provided at the base with COBB'S "pseudobulb". Female genital tube paired, symmetrical. Vulva situated fairly posterior to the middle of the body. Male genital tube paired. Spicule plumpy, without accessory piece. Male with a ventral row of small papilliform supplementary organs extending from the front of the anus to near the end of oesophagus. Caudal glands and spinneret absent. Tail of the male similar in form and size to that of the female.

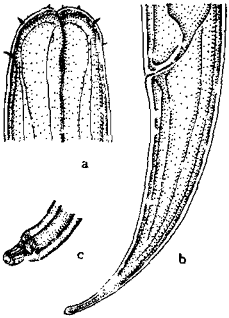
4. *Tripyla papillata* BÜTSCHLI, 1873.

Text-fig. 4.

No=46 ♀10 ♂3 Larvae 33 (Sex. coef.=30 n=13)

♀ L=2.43–3.05 mm. $a=22.3-28.1$ $\beta=4.7-5.6$ $\gamma=6.5-7.3$
V=52.3–56.1%

♂ L=2.45–2.69 mm. $a=20.4-25.3$ $\beta=4.6-5.1$ $\gamma=6.3-7.0$



Text-fig. 4. *Tripyla papillata* BÜTSCHLI.
a. Anterior part of the female.
b. Posterior part of the female.
c. Terminus of the female.

Body fairly slender, tapering slightly anteriorly and extremely posteriorly. Cuticle transversely striated, without bristle. Lateral organ invisible. Anterior end indistinct, with 3 lips and some bristle-like papillae. Oesophagus slightly swollen posteriorly, with a large lobed oesophageal gland. Female genital tube paired, symmetrical. Vulva located slighly behind the middle of the body. Male genital tube paired. Spicule plumpy, presenting a central thickening, but without accessory piece. A mid-ventral row of preanal papillae present. Tail almost similar in both sexes, gradually tapering backwards to end in a somewhat rounded terminus. Caudal glands and spinneret present.

II. FAM. Trilobidae MICOLETZKY, 1922.

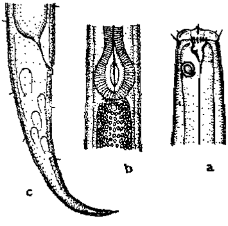
5. *Cyatholaimus ruricola* DE MAN, 1880.

Text-fig. 5.

No=9 ♀ 5 Larvae 4

♀ L=0.45–0.58 mm. α=20.1–25.3 β=5.8=6.3 γ=7.2–8.3
V=43.1–46.5 %

Body rather slender, tapering slightly anteriorly and extremely posteriorly. Cuticle traversed by fine transverse striae. Anterior end not truncated, with fairly high rounded lips and 6 fine fairly long cephalic setae. Lateral organ spiral at the base of the pharynx. Pharynx chitinized, very long, marked with a small dorsal tooth and some longitudinal chitinous stripes. Vulva situated slightly in front of the middle of the body. Tail fairly long, with caudal glands and spinneret.



Text-fig. 5. *Cyatholaimus ruricola* DE MAN.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

6. *Monhystera filiformis* BASTIAN, 1865.

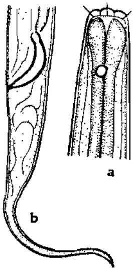
Text-fig. 6.

No=16 ♀ 7 ♂ 4 Larvae 5 (Sex. coef.=57.1 n=11)

♀ L=0.59–0.87 mm. α=21.3–26.2 β=4.2–5.0 γ=4.5–5.3

V=59.3–63.1%

♂ L=0.56–0.63 mm. α=22.4–25.1 β=4.6–5.2 γ=5.0–5.5



Text fig. 6. *Monhystera filiformis* BASTIAN.
a. Anterior part of the male.
b. Posterior part of the male.

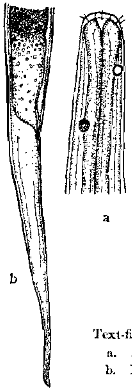
Body fairly slender, tapering slightly forwards and extremely backwards. Cuticle unstriated, with fine bristles, but without wing. Anterior end with indistinct lips and 6 fairly short cephalic setae. Lateral organ usually circular, about twice as wide as the head at the level far behind the anterior end. Buccal capsule small, cup-shaped, lined with a thin wall continuous *with that of the oesophagus;* buccal cavity funnel-shaped. Oesophagus cylindroid, usually swollen posteriorly, with 3 distinct bulbs. Ocelli absent. Intestinal cells usually of a light blue colour. Female genital tube single, prevulvar. Vulva situated behind the middle of the body. Male genital tube single. Spicule slender, without accessory piece. Male with a submedian ventral row of preanal papillae. Postanal papillae also present. Tail almost identical in form and size in both sexes, bearing caudal glands and spinneret.

7. *Monhystera paludicola* DE MAN, 1880.

Text-fig. 7.

No=1 ♀ 1

♀ L=0.59 mm. α=24.6 β=4.2. γ=6.5 V=74.6%



Body fairly slender, tapering slightly forwards and extremely backwards. Cuticle unstriated, without bristle and wing. Anterior end without any trace of lip, but with 6 short cephalic setae. Pharynx very similar to that of *M. filiformis* BASTIAN. Lateral organ circular, almost as wide as the head at the level far behind the anterior end. Ocelli present. Without oesophageal end-bulb. Intestinal cells usually dark. Tail not so long, with caudal glands and spinneret.

Text-fig. 7. *Monhystera paludicola* DE MAN.
a. Anterior part of the female.
b. Posterior part of the female.

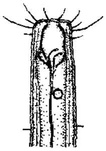
8. *Monhystera agilis* DE MAN, 1880.

Text-fig. 8.

No=1 Larva 1

Larva L=1.62 mm. $\alpha=60.0$ $\beta=4.6$ $\gamma=2.9$

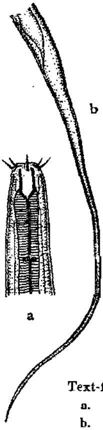
Body extremely slender, tapering very slightly forwards and extremely backwards. Cuticle striated, with fine transverse striae, bearing distinct bristles here and there. Anterior end without any trace of lip, but with 10 long cephalic setae, of which 6 are larger than the rest. Lateral organ small and circular. Pharynx large, spacious, *Mononchus*-like. Oesophagus cylindroid, slightly swollen behind, without bulb. Intestinal cells of a light blue colour. Tail extremely long, thread-like, ending in an extremely fine hair-like terminus. Movement highly active.



Text fig. 8. *Monhystera agilis* DE MAN. Anterior part of the larva.

9. *Prismatolaimus dolichulus* DE MAN, 1880.

Text-fig. 9.



No=3 ♀ 1 Larvae 2

♀ L=0.48 mm. a=41.2 β=4.4 γ=2.6

V=45.2%

Body very slender, tapering slightly forwards and extremely backwards. Cuticle finely striated, without bristle and papilla. Head rounded, indistinct, without lip, but with 6 strong submedian cephalic setae. Lateral organ lineal, extending farther back. Pharynx short, prismatic, unarmed. Oesophagus slightly swollen posteriorly, without bulb. Female genital tube paired, symmetrical. Vulva located in front of the middle of the body. Tail extremely long, with an extremely fine hair-like terminus. Caudal glands present.

Text-fig. 9. *Prismatolaimus dolichulus* DE MAN.
a. Anterior part of the female.
b. Posterior part of the female.

10. *Trilobus gracilis* BASTIAN, 1865.

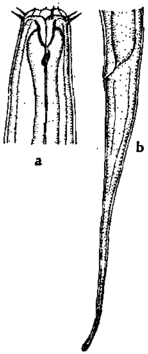
Text-fig. 10.

No=2 ♀ 2

$♀_1$ L=1.55 mm. a=31.0 β=4.8 γ=6.4. V=46.2%

$♀_2$ L=1.65 mm. a=23.5 β=5.0 γ=13.5 V=48.5%

Body fairly slender, tapering towards both ends, especially backwards. Cuticle unstriated, often with fine bristles on the body surface. Lateral organ invisible. Anterior end indistinct, with rudimentary lips and



Text-fig. 10. *Trilobus gracilis* BASTIAN.
a. Anterior part of the female.
b. Posterior part of the female.

6 short cephalic setae. Pharynx spacious, funnel-shaped, lined with the chitinous wall, wholly destitute of tooth or local thickening. Oesophagus with tooth-like processes near the anterior end, swollen at the posterior end, with three-lobed oesophageal glands. Tail elongate-slender, gradually tapering to a rounded terminus. Female genital tube paired, symmetrical. Vulva located slightly in front of the middle of the body.

The specimen before me is clearly distinguishable from the form dealt with by STEFANSKI, in which the pharynx is not spacious and is entirely devoid of tooth-like process near the anterior end of the oesophagus, and the tail is extremely short, conical and ends in a somewhat pointed terminus.

III. FAM. Rhabditidae MICOLETZKY, 1922.

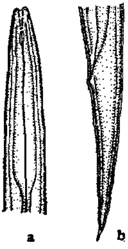
11. *Cephalobus elongatus* DE MAN, 1880.

Text-fig. 11.

No=4 ♀ 2 Larvae 2

♀ L=0.66−0.69 mm. $a=22.0-24.1$ $\beta=3.9-4.2$ $\gamma=9.4-11.3$
V=63.1−65.0%

Body fairly slender, tapering towards both ends, especially backwards. Cuticle apparently striated, without bristle, but with a distinct wing. Lateral organ invisible. Anterior end consisting of 3 low rounded lips, without cephalic seta and papilla. Pharynx elongate-cylindroid, somewhat slender at the proximal end. Pharynx with the wall chitinized here and there. Oesophagus widely cylindroid in the distal half, suddenly tapering towards the proximal-end bulb,



Text-fig. 11. *Cephalobus elongatus* DE MAN.
a. Anterior part of the female.
b. Posterior part of the female.

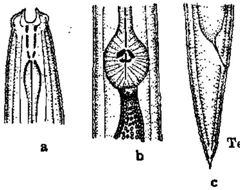
bearing a strong bulbular apparatus. Excretory pore distinctly recognized at a point a little in front of the nerve-ring. Female genital tube single, prevulvar. Tail plumpy, conical, pointed at the end.

12. *Cephalobus emarginatus* DE MAN, 1880.

Text-fig. 12.

No=1 ♀ 1
♀ L=0.73 mm. $\alpha=24.3$ $\beta=4.0$ $\gamma=16.2$ V=61.6%

This species represented by one individual only is very closely related to *C. elongatus* DE MAN, but differs from it in having the anterior end which is deeply and laterally cut off, forming 2 pointed ends.



Text fig. 12. *Cephalobus emarginatus* DE MAN.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

13. *Cephalobus persegnis* BASTIAN, 1865.

Text-fig. 13.

No=2 ♀ 1 Larva 1
♀ L=0.55 mm. $\alpha=18.3$ $\beta=3.6$ $\gamma=17.1$
V=63.6%

Body fairly plumpy, tapering towards both ends. Cuticle striated, with a fairly wide wing. Anterior end with 3 fairly low rounded lips, but without papilla. Pharynx not so long, cylindroid, somewhat narrowing at the base. Oesophagus widely developed in the distal half, gradually narrowing towards the front of the true bulb. Female genital tube unpaired, prevulvar. Vulva situated near the posterior end of the middle third of the body. Tail plumpy, conical, ending in a rounded terminus.

Text-fig. 13. *Cephalobus persegnis* BASTIAN.
a. Anterior part of the female.
b. Posterior part of the female.

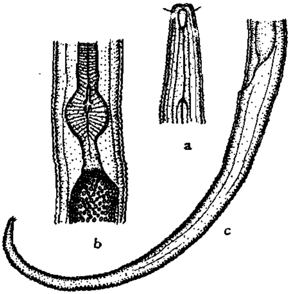
14. *Chronogaster gracilis* COBB, 1913.

Text-fig. 14.

No=9 ♀ 5 Larvae 4

♀ L=1.08–1.53 mm. α=36.0–43.1 β=4.3–4.9 γ=6.4–8.5

V=50.4–53.7%



Text-fig. 14. *Chronogaster gracilis* COBB.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

Body slender, tapering towards both ends. Cuticle apparently traversed by fine transverse striae, without bristle and ala. Anterior end indistinct, with 3 rudimentary lips and 4 submedian cephalic setae. Lateral organ trapezoid, open behind, but almost indistinct. Pharynx funnel-shaped, with the chitinous wall. Oesophagus with a posterior bulb bounded by a long constricted part from the intestine. Female genital tube unpaired, prevulvar. Vulva situated slightly behind the middle of the body. Tail elongate-slender, conical, ending in a rounded terminus, bearing caudal glands and papilliform spinnerets.

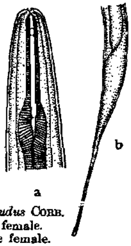
15. *Chriptonchus nudus* COBB, 1913.

Text-fig. 15.

No=8 ♀ 1 Larvae 7

♀ L=1.82 mm. α=42.5 β=3.8 γ=9.5 V=52.1%

Body very slender, tapering slightly forwards and extremely backwards. Cuticle naked, finely striated. Anterior end presenting 6 indistinct lips with a crown of very small innervated papillae. Lateral organ *Plectus*-like, near the head. Pharynx very long, tubular, with some small dorsal teeth at the base. Oesophagus without bulb. Female genital tube unpaired, prevulvar. Vulva situated slightly behind the middle of the body. Tail fairly long and slender. Caudal glands and spinneret present.



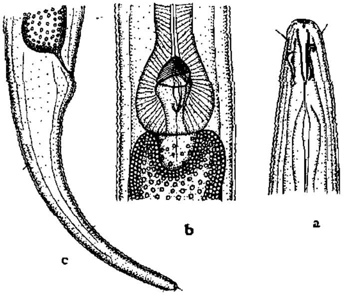
Text-fig. 15. *Criptonchus nudus* COBB.
a. Anterior part of the female.
b. Posterior part of the female.

16. *Plectus cirratus* BASTIAN, 1865.

Text-fig. 16.

No=28 ♀ 16 Larvae 12

♀ L=0.89−1.37 mm. α=23.3−27.4 β=3.9−4.5 γ=9.3−11.4
V=49.6−53.4%



Text-fig. 16. *Plectus cirratus* BASTIAN.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

Body fairly slender, tapering towards both ends, especially backwards. Cuticle striated, with a fairly wide wing. Anterior end distinct, with 6 rounded lips and 6 short cephalic setae slightly behind the end. Lateral organ elliptical, open behind, slightly in front of the middle of pharynx. Pharynx elongate, with the chitinous wall, gradually tapering backwards. Oesophagus cylindroid, somewhat slender near the middle, posteriorly exhibiting a large swelling which contains a very strong bulbular apparatus. Female genital tube paired, symmetrical. Vulva situated near the middle of the body. Tail fairly long, gradually tapering backwards. Caudal glands and spinneret present.

17. *Plectus multitubiferus* sp. nov.

Text-fig. 17.

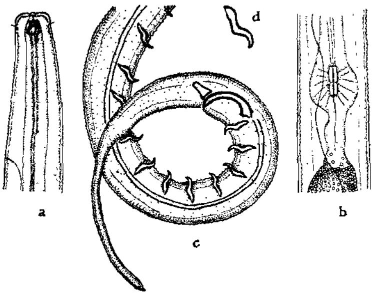
No=5 ♀ 3 ♂ 2 (Sex. coef.=66.5 n=5)

♀ L=1.05 (0.94−1.17) mm. α=33.0 (31.3−34.0) β=6.5 (6.4−6.6)
γ=6.2 (5.3−7.2) V=47.4 (47.3−47.5)%

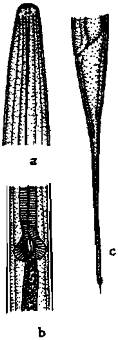
♂ L=1.01 (0.97−1.04) mm. α=35.3 (34.6−35.9) β=6.0 (5.0−60)
γ=6.1 (6.0−6.1)

Body slender, tapering very slightly forwards and extremely backwards. Cuticle thin, transparent, colourless, traversed by fine transverse striae, provided with a pair of narrow but distinct wings extending from the middle of the oesophagus to near the anus. No hair on the surface of body. Anterior end with 3 (perhaps 6) lips and 4 extremely minute cephalic setae slightly behind the end. Lateral organ elliptical, open below, situated slightly behind the cephalic setae. Pharynx tubular, very long and narrow. Frequently there can be seen 3 chitinous thickenings near the upper part of the pharynx. Oesophagus elongate-cylindroid, with an oval swelling posteriorly which contains a strong bulbular apparatus, consisting of two sets of small rectangular chitinous thickenings. Ventral gland present, with a distinct pore. Female genital tube paired, symmetrical. Vulva lying a little anterior to the middle of the body. Male genital tube unpaired. Spicule slender, strongly acute, provided with a slightly pointed accessory piece. In front of the anus there is a ventral row of 13 tubular protrusile supplementary organs, which begin at the proximal end of the spicule and extend over a space almost twice as long as the tail. Each organ is about half as long as the body theth and is curved in its distal half. Two postanal papillae are present dors.nt, of the middle of the tail. Tail abruptly tapering just in front of Fema. is, ending in a cylindroid terminus, almost identical in form and slightly oth sexes. Caudal glands and spinneret present.
and slen

new species is most closely allied in the female to *Plectus granu-* TIAN and in the male to *Aphanolaimus spiriferus* COBB and *A.* B especially in the posterior half of the body



Text-fig. 17. *Plectus multitubiferus* sp. nov.
a. Anterior part of the male.
b. Oesophagus-end region of the male.
c. Posterior part of the male.
d. One of the tubular protrusile supplementary organs.



Text-fig. 18. *Rhabdolaimus aquaticus* DE MAN.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

18. *Rhabdolaimus aquaticus* DE MAN, 1880.

Text-fig. 18.

No=8 ♀ 3 Larvae 5
♀ L=0.61–0.66 mm. $\alpha=23.2-26.4$
$\beta=4.4-4.7$ $\gamma=4.1-4.4$
V=45.4–47.3%

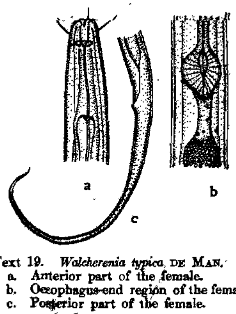
Body fairly slender, tapering slightly anteriorly and extremely posteriorly. Cuticle naked, presenting fine transverse striations interiorly. Anterior end bluntly rounded, without lip, papilla or bristle. Lateral organ small, inconspicuous. Pharynx long, narrow, supported by 3 slender chitinous rods which are thought to be labial toothlets. Oesophagus with a posterior muscular bulb. Female genital tube paired, symmetrical. Vulva situated slightly anterior to the middle of the body. Tail elongate-slender, gradually tapering backwards. Caudal glands and spinneret present.

19. *Walcherenia typica* DE MAN, 1921.

Text-fig. 19.

No=1 ♀ 1
♀ L=1.21 mm. $\alpha=40.3$ $\beta=5.5$ $\gamma=5.8$ V=47.1%

Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle striated, without papilla and bristle. Anterior end without lip, but with 4 long cephalic setae behind the end. Lateral organ fairly distinct, transversely elliptical, open behind, located near the base of the pharynx. Pharynx cylindroid, abruptly constricted behind. Oesophagus with a posterior bulb, as in *Plectus*. Female genital tube unpaired, prevulvar. Vulva situated in front of the middle of the body. Tail very long, thread-



Text 19. *Walcherenia typica* DE MAN.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

like, ending in a hair-like terminus. Caudal glands and excretory pore indistinct.

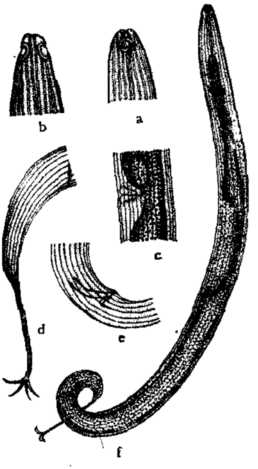
20. *Pseudoaulolaimus anchilocaudatus* gen. et sp. nov.

Text-fig. 20.

No=121 ♀ 51 ♂ 33 Larvae 27 (Sex. coef.=64.7 n=84)

♀ L=0.95 (0.87–1.05) mm. α=30.8 (29.0–32.6) β=10.6 (10.0–11.6)
γ=5.4 (5.1–5.8) V=38.0 (35.5–40.0)%

♂ L=0.98 (0.82–1.15) mm. α=31.9 (30.3–32.6) β=10.3 (9.6–11.5)
γ=5.6 (5.4–6.0)



Text-fig. 20. *Pseudoaulolaimus anchilocaudatus* gen. et sp. nov.
a. Side view of the head region.
b. Dorso-median view of the head region.
c. Vulvar region of the female.
d. Posterior part of the female.
e. Anal region of the male.
f. Total view of the male.

Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle thin, transparent, colourless, highly marked with many distinct longitudinal and very fine transverse striae. The longitudinal ones are arranged in pairs near the lateral organ and amount to about 24 (16–32) in all, extending from the middle of the neck to the anus. As they proceed towards the tail end, they are gradually fused with one another to form four cuticular ribs and finally form 4 anchor-shaped terminal branches. Some of these branches are often subdivided once or more. Anterior end naked, slightly concave at the apex, with 3 low rounded lips. Lateral organ spiral, situated near the anterior end, half as wide as the body. Pharynx cylindroid, elongate-slender, half or more as long as the oesophagus. Oesophagus short, cylindroid, without bulb posteriorly. Lining of the oesophagus distinct. Intestine not separated from the oesophagus by a distinct constriction, about three-fourths as wide as the body, of a

light yellowish colour, on account of numerous fine coloured granules in the cells. Prerectum not distinct. Female genital tube paired, symmetrical. Vulva situated in front of the middle of the body. Ova elliptical. Male genital tube not distinct. Spicule short, accute, provided with an accessory piece which is bluntly pointed and abruptly bends backwards, as in Text-fig. 20e. There is no male supplementary organ or papilla in front of the anus. Tail tapering from the front of the anus to near the middle, thence suddenly becoming narrower and cylindroid, ending in 4 anchor-shaped terminal branches. Form and size of the tail almost identical in both sexes. Caudal glands not determined.

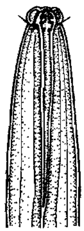
This new genus seems to be most closely related to *Aulolaimus* DE MAN, but differs from it by the following main points:—1) Lateral organ very distinctly developed in spiral form; 2) at the anterior end there can be seen 3 fairly distinct lips; 3) cuticle marked with many conspicuous longitudinal striae; 4) pharynx half or more as long as the oesophagus; 5) preanal papillae entirely absent in the male; 6) vulva situated apparently in front of the middle of the body; 7) tail ending in four or more anchor-shaped terminal branches.

So far as examinations go, the appearance of this species after irrigation amounts to 92.8% in frequency; consequently it may be said to freqnent water-saturated soil or fresh water more than in wet soil. The movement of this worm is very slow.

IV. FAM. *Oncholaimidae* BAYLIS & DAUBNEY, 1926.

21. *Ironus ignavus* BASTIAN, 1865.

Text-fig. 21.



Text-fig. 21. *Ironus ignavus* BASTIAN. Anterior part

No=96 ♀ 32 Larvae 64

♀ L=1.37–2.10 mm. α=37.4–41.2

β=4.1–4.7 γ=11.4–13.5

V=47.6–50.9%

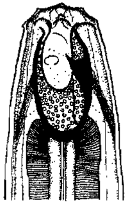
Body slender, tapering towards both ends, especially posteriorly. Cuticle unstriated, without papilla and bristle. Anterior end with 3 lips and 4 submedian cephalic setae slightly behind the end. Pharynx elongate-slender, with 3 strong hook-shaped teeth at the anterior end as well as with 3 toothlets a little behind the end. Oesophagus

paired, symmetrical. Vulva situated near the middle of the body. Tail long, thread-like, ending in a hair-like terminus. Caudal glands present.

22. *Mononchus macrostoma* BASTIAN, 1865.

Text-fig. 22.

No=51 ♀ 39 Larvae 12
♀ L=1.83–2.05 mm. $\alpha=27.8-33.3$ $\beta=3.9-4.2$ $\gamma=8.9-11.4$
V=49.6–55.4%



Text-fig. 22. *Mononchus macrostoma* BASTIAN. Head region of the female.

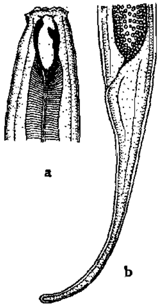
Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle unstriated, without bristle. Labial region not distinct, with bristle-like papillae. Lateral organ oval, very small, almost indistinct. Pharynx large, spacious, oval, about half as wide as the body at the corresponding level, containing a dorsal tooth which slightly projects from the middle of the chitinous wall into the lumen. Oesophagus without bulb, but slightly swollen posteriorly. Female genital tube paired, symmetrical. Vulva located near the middle of the body. Tail rather long, cylindroid in the posterior part, ending in a somewhat expanded terminus.

23. *Mononchus tunbrigdensis* BASTIAN, 1865.

Text-fig. 23.

No=46 ♀ 34 Larvae 12
♀ L=1.15–1.46 mm. $\alpha=27.7-30.1$
$\beta=4.1-4.4$ $\gamma=8.4-9.3$
V=52.6–56.0%

This species is most closely allied to *M. macrostoma* BASTIAN, but it differs from this in its smaller size as well as in the possession of the pharynx which is about one-third as wide as the body at the corresponding level and bears a dorsal tooth projecting from the front of the middle of the wall into the lumen.



Text-fig. 23. *Mononchus tunbrigdensis* BASTIAN.
a. Anterior part of the female.
b. Posterior part of the female.

24. *Oncholaimus punctatus* COBB, 1914.

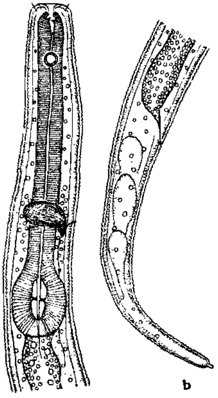
No=1 Larva 1

Unfortunately the proportional numbers could not be determined.

Body fairly slender, tapering slightly forwards and extremely backwards. Cuticle unstriated. Anterior end distinct, with 6 well-developed lips and fairly distinct chitinous frame-work. Slightly behind the lips are found an anterior circlet of papillae and a posterior one of papilla-like cephalic setae. Lateral organ transversely oval. Pharynx spacious, with the chitinous wall as in *Mononchus*, containing a dorsal and two subventral teeth which carry the ducts of the oesophageal glands. Oesophagus gradually swollen behind, without bulb. Tail gradually tapering from the anus to the posterior limit of its anterior one-third and thence becoming cylindroid up to the terminus. Two distinct ventral caudal papillae near the anterior one-fourth of the tail. Caudal glands and spinneret present.

25. *Chromadola fluviatilis* COBB, 1914.

Text-fig. 24.



Text-fig. 24. *Chromadola fluviatilis* COBB.
a. Anterior part of the female.
b. Posterior part of the female.

No=1 ♀ 1

♀ L=0.76 mm. $\alpha=25.3$ $\beta=6.33$ $\gamma=36.2$ V=43.4%

Body fairly slender, slightly tapering towards the anterior end. Cuticle traversed by very fine transverse striae. Anterior end rounded, with a circlet of 4 cephalic setae slightly behind the end. Lateral organ distinct, circular, located rather far behind the pharyngeal base. Pharynx prismoid, armed with minute somewhat inwardly pointed teeth, of which the first is dorsal, the second ventral just behind the base of the lips and the third dorsal but inconspicuous at the base. Oesophagus wide, cylindroid up to the pyriform cardiac bulb containing a distinct chitinous bulbular apparatus. The excretory pore seems to be located opposite to the nerve-ring. Female

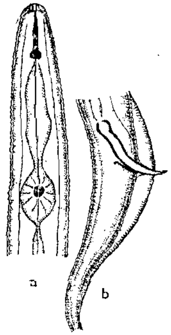
genital tube paired, symmetrical Tail conoid, tapering from the anus towards the terminus. Caudal glands and spinneret present.

V. FAM. Anguillulinidae BAYLIS & DAUBNEY, 1926.

26. *Tylenchus gracilis* DE MAN, 1880.

Text-fig. 25.

No=478 ♀ 92 ♂ 25 Larvae 361 (Sex. coef.=27.2 n=117)
♀ L=2.75–3.53 mm. α=72.3–85.0 β'=22.9–35.3 γ=24.2–27.6
V=49.4–58.6%
♂ L=2.25–3.21 mm. α=75.0–83.1 β'=21.3–32.1 γ=22.5–26.3



Text-fig. 25. *Tylenchus gracilis* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

Body extremely slender, tapering towards both ends. Cuticle apparently striated, without bristle. Lateral organ not determined. Anterior end somewhat semispherical, without lip and cephalic seta, but with complex chitinous framework serving as a guiding ring for the spear. Spear very strong, 3-lobed at the end, its length about one-fifth as long as the distance from the head to the front of the median sucking bulb. Posterior part of the oesophagus behind the bulb and the beginning of the intestine not distinct. Female genital tube paired, symmetrical. Vulva situated slightly behind the middle of the body. Male genital tube single. Spicule slightly plumpy, provided with a short somewhat curved accessory piece. Tail short, somewhat cylindroid, but gradually tapering backwards, ending in a sharply pointed terminus. Form and size of the tail almost identical in both sexes. Bursa present in the male, wrapping the tail completely, with a bursal papilla slightly behind the middle of the tail.

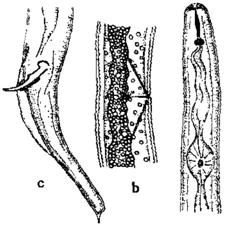
27. *Tylenchus apapillatus* sp. nov.

Text-fig. 26.

No=56 ♀ 33 ♂ 7 Larvae 16 (Sex. coef.=21.2 n=40)

♀ L=1.17 (0.93–1.50) mm. α=55.0 (50.0–60.0)
β'=20.3 (17.4–25.0) γ=17.7 (16.3–25.0)
V=53.3 (50.5–58.0)%
♂ L=1.17 (1.02–1.32) mm. α=53.3 (52.8–53.7)
β'=22.1 (21.1–24.3) γ=20.8 (20.4–21.3)

Body extremely slender, tapering towards both ends. Cuticle thin, transparent, colourless, traversed by fine transverse striae, with a fairly distinct wing extending almost throughout the body. Lateral organ not found. Anterior end semispherical, without lip and cephalic seta, but with complex chitinous frame-work serving as a guiding ring for the spear. Spear strong in both sexes, 3-lobed at the base, as long as the body at the level of the base. Median swollen bulb oval, containing a bulbular apparatus which consists of 3 small chitinous thickenings of a crescentic shape. Posterior part of the oesophagus not distinct. Excretory pore distinct, located fairly far behind the median swollen bulb. Intestine colourless, filled with numerous fairly large granules. Female genital tube paired, symmetrical. Vulva situated slightly behind the middle of the body. Male genital tube unpaired. Spicule rather slender, provided with an accessory piece which is about half as long as the spicule. Bursa not wrapping the tail completely. No papillae on the bursa. Tail gradually tapering from the front of the anus to near the posterior end of its anterior third, thence becoming cylindroid up to the pointed terminus. Tail almost identical in shape in both sexes, a little longer in the female than in the male.



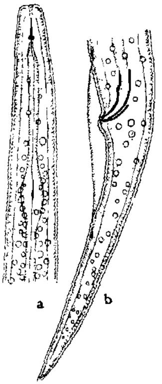
Text-fig. 26. *Tylenchus apapillatus* sp. nov.
a. Anterior part of the male.
b. Vulvar region of the female.
c. Posterior part of the male.

This species is most closely related to *Tylenchus gracilis* DE MAN, but it can be distinguished from this by its smaller size (1.17 mm. : 3 mm.), the possession of a distinct wing and the bursa not wrapping the tail completely, and the absence of papillae on the bursa.

28 *Tylenchus intermedius* DE MAN, 1880.

Text-fig. 27.

No=23 ♀ 6 ♂ 4 Larvae 13 (Sex. coef.=66.6 n=10)



Text-fig. 27. *Tylenchus intermedius* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

♀ $L = 0.8 - 1.10$ mm. $\alpha = 36.6 - 47.2$
$\beta = 6.1 - 7.5$ $\gamma = 6.5 - 8.1$
$V = 75.0 - 80.0\%$
♂ $L = 0.82 - 1.05$ mm. $\alpha = 33.7 - 42.1$
$\beta = 4.8 - 5.2$ $\gamma = 10.3 - 13.7$

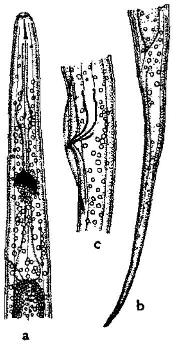
Body slender, tapering slightly forwards and extremely backwards. Cuticle finely striated, without bristle. Anterior end semispherical, slightly truncated at the extremity, entirely destitute of lip, cephalic seta and chitinous framework. Spear small, feeble, with 3 small knobs posteriorly. Oesophagus distinct, with a small oval median sucking bulb and enlarged to form a pocket-like shape at its posterior end. Female genital tube unpaired, prevulvar. Vulva situated far behind the middle of the body. Male genital tube single. Spicule somewhat plumpy, slightly curved, provided with a short slender accessory piece. Tail long, gradually tapering backwards, almost identical in form in both sexes but somewhat longer in the female than in the male. Bursa present in the male, not wrapping the tail completely, usually without bursal papilla.

29. *Tylenchus filiformis* BÜTSCHLI, 1873.

Text-fig. 28.

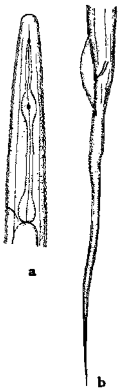
No=29 ♀ 12 ♂ 6 Larvae 11
(Sex. coef. = 50 n = 18)
♀ $L = 1.10 - 1.27$ mm. $\alpha = 36.6 - 42.3$
$\beta = 6.5 - 7.1$ $\gamma = 4.6 - 5.3$
$V = 61.4 - 62.6\%$
♂ $L = 1.02 - 1.31$ mm. $\alpha = 39.4 - 45.2$
$\beta = 6.9 - 7.5$ $\gamma = 6.7 - 7.0$

Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle very finely striated, with a pair of fairly broad wings. Anterior end somewhat semispherical, slightly concave at the extremity, without lip and



Text-fig. 28. *Tylenchus filiformis* BÜTSCHLI.
a. Anterior part of the female.
b. Posterior part of the female.
c. Anal region of the male.

chitinous frame-work. Spear very small, feeble, 3-lobed at the posterior end. Oesophagus with a fairly small median bulb and enlarged at the posterior end. Female genital tube unpaired, prevulvar. Vulva situated near the middle third of the body. Male genital tube single. Spicule somewhat slender, slightly curved, with a short accessory piece. Tail very long, thread-like in both sexes. Bursa wrapping the tail partly in the male, without bursal papilla.



Text-fig. 29. *Tylenchus leptosoma* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

30. *Tylenchus leptosoma* DE MAN, 1880.

Text-fig. 29.

No=2 ♂ 1 Larva 1
♂ L=0.9 mm. $\alpha=40.9$ $\beta=6.0$ $\gamma=6.0$

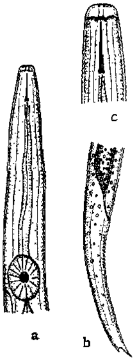
Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle with very fine striations. Anterior end rounded, completely naked, without lip and chitinous frame-work. Oral spear weak, with posterior knobs. Oesophagus distinct, with a small oval median bulb, enlarged posteriorly. Male genital tube single. Spicule shortly slender, accessory piece invisible. Tail extremely long, gradually tapering backwards, ending in a fine hair-like terminus. Bursa very small, somewhat semicircular, without bursal papilla.

31. *Aphelenchus bicaudatus* sp. nov.

Text-fig. 30.

No=18 ♀ 18
♀ L=0.43 (0.38–0.47) mm. $\alpha=31.5$ (31.3–31.7) $\beta'=7.4$ (6.8–8 4)
$\gamma=10.6$ (9.4–12.6) V=70.4 (61.7–90.2)%

Body slender, tapering slightly anteriorly and fairly posteriorly. Cuticle thin, transparent, colourless, traversed by extremely fine transverse striae, without bristle. No wing on the body surface. Anterior end naked, somewhat rounded, without lip, but with 3 small chitinous pieces at the extremity which serve as a guiding ring for the spear. Knob-like chitinous



Text-fig. 30. *Aphelenchus bicaudatus* sp. nov.
a. Anterior part of the female.
b. Posterior part of the female.
c. Head region of the female.

cap divided into three parts, as in Text-fig. 30c. Oesophagus distinct before but ambiguous behind the median swelling which is large and rounded. Intestine colourless, filled with numerous small granules up to near the anus. Female genital tube unpaired, prevulvar. Vulva located near the third one-fourth of the body. Tail gradually tapering to the terminus which is bifurcated in different lengths.

This species is apparently distinguished from other species of this genus by its smaller size and bifurcated tail. Male unknown.

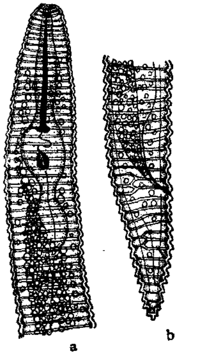
32. *Criconema komabaensis* sp. nov.

Text-fig. 31.

No=2 ♀ 2

♀ 1 L=0.43 mm.	α=13.9	β=4.3
γ=14.3 ?	V=86.0%	Rn=138
♀ 2 L=0.44 mm.	α=13.4	β=4.4
γ=16.3 ?	V=90.0%	Rn=139

Body plumpy, tapering towards both ends, considerably posteriorly. Cuticle thick, transparent, colourless, traversed by about 140 transverse striae, of which 22 or 23 occur extending from the anterior end to the base of spear, and 16-17 from the vulva to the terminus. These transverse striae are connected together, partly to form longitudinal stripes. Anterior end truncated, with small dentated lips. Oral spear very long, powerful, ending in 3 strong knots at the base. Its lumen leads to that of the oesophagus which is swollen into two bulbs. The anterior bulb is larger and contains the base of the spear as well as a strong bulbular



Text-fig. 31. *Criconema komabaensis* sp. nov.
a. Anterior part of the female.
b. Posterior part of the female

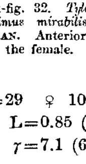
apparatus, consisting of 3 small half-moon-shaped chitinous thickenings as in the case of *Tylenchus*, *Aphelenchus*, etc. The posterior bulb is a great deal smaller and merges in the narrow posterior part of the oesophagus posteriorly. Intestine filled with numerous fairly large granules. Female genital tube unpaired, prevulvar. Vulva very distinct, situated far behind. Anus too ambiguous for determining its position distinctly. Tail made up of segments, telescopic, gradually tapering backwards.

At a glance, this species appears to be very much like *Criconema rusticus* MICOLETZKY but differs from it by the following main points:—1) Cuticle marked with transverse striae more numerous than that of *C. rusticus* (ca. 140 : ca. 100 in all; 22–23 : 12–19 from the anterior end to the base of the spear; 16–17 : 6–10 from the vulva to the terminus); 2) the first oesophageal bulb contains a well developed bulbular apparatus made up of 3 small half-moon-shaped chitinous thickenings.

33. *Tylencholaimus mirabilis* BÜTSCHLI, 1873.

Text-fig. 32.

No=2 ♀ 1 Larva 1
♀ L=1.52 mm. α=31.3 β=4.3 γ=32.3 V=50.6%



Text-fig. 32. *Tylencholaimus mirabilis* DE MAN. Anterior end of the female.

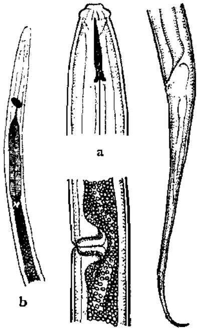
Body slender, slightly tapering towards both ends. Cuticle unstriated, without ala and bristle. Anterior end truncated, disk-like shaped, with a crown of 6 innervated papillae. Oral spear short, strong, trifurcated at the base. Oesophagus dorylaimoid, enlarged in the posterior half. Female genital tube unpaired, prevulvar. Vulva situated near the middle of the body. Tail short, conoid, ending in a blunt terminus.

34. *Tylencholaimus longicaudatus* sp. nov.

Text-fig. 33.

No=29 ♀ 10 Larvae 19
♀ L=0.85 (0.81–0.88) mm. α=30.8 (28.5–33.1) β=3.5 (3.4–3.7)
γ=7.1 (6.9–7.3) V=58.5 (54.1–61.7)%

Body fairly slender, tapering very slightly anteriorly and extremely posteriorly. Cuticle naked, externally unstriated, but traversed by extremely fine internal striations. Anterior end truncated, disk-like, with a circle of



Text-fig. 33. *Tylencholaimus longicaudatus* sp. nov.
a. Anterior end of the female.
b. Anterior part of the female.
c. Vulvar region of the female.
d. Posterior part of the female.

6 innervated papillae. Spear short, about one-fourteenth the oesophagus, fairly strong, trifurcated posteriorly. Oesophagus extremely narrowed in the distal half, thence suddenly thickened backwards. Lining of the oesophagus very distinct only in the proximal half. Ventral gland and excretory pore not found. Cardia distinct, conical, disposed posteriorly from the oesophagus into the intestine. Intestine always brownish. Prerectum distinct, as long as the tail. Female genital tube unpaired, prevulvar. Vulva situated slightly behind the middle of the body. Tail very long, gradually tapering to the pointed terminus.

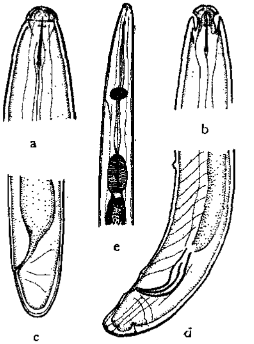
This species is very much like *Tylencholaimus mirabilis* BÜTSCHLI and *T. minimus* DE MAN but it is distinguished from them and others belonging to this genus by the possession of a very long tail.

35. *Tylencholaimus bryophilus* sp. nov.

Text-fig. 34.

No=48 ♀ 15 ♂ 9 Larvae 24 (Sex. coef.=60 n=24)
♀ L=1.49 (1.23–1.75) mm. α=34.6 (32.2–36.9) β=7.3 (7.0–7.5) γ=49.5 (47.1–53.2) V=55.4 (52.7–56.8)%
♀ L=1.44 (1.36–1.57) mm. α=35.0 (34.0–37.2) β=6.9 (6.7–7.1) γ=51.1 (48.3–54.4) Pn=1+(6–7)

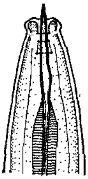
Body slender, moderately tapering towards both ends. Cuticle rather thick, naked, traversed by very fine internal transverse striae. Anterior end not disk-like shaped, nearly semispherical, with 2 crowns of 6 innervated papillae. Oral spear very short, about one-twelfth as long as the oesephagus, slender, trifurcated at its base. Oesophagus short, extremely narrowed in the anterior portion. At the beginning of the posterior fifth, it suddenly expands to form a bulb, much as in *Tylencholaimus affinis* BRAKENHOFF. Nerve ring slightly in front of the middle of the oesophagus. Excretory pore distinct, slightly behind the nerve ring. Cardia conical, well developed,



Text-fig. 34. *Tylencholaimus bryophilus* sp. nov.
a. Side view of the head region.
b. Dorso-median view of the head region.
c. Posterior part of the female.
d. Posterior part of the male.
e. Anterior part of the female.

extending from the end of the oesophagus into the intestine. Intestine of the normal type in structure. Prerectum always distinct, about four times in the male, and three times in the female, as long as the body width at the corresponding level. Female genital tube unpaired, prevulvar. Vulva a little behind the middle of the body. Male genital tube single. Spicule slender, curved, with a central thickening, but without accessory piece. Male with an anal papilla and 6–7 preanal papillae arranged in the mid-ventral row, extending to near the beginning of the prerectum. Tail short, plumpy, rounded at end in both sexes, with 4–6 innervated papillae distinctly in the male but very obscurely in the female. At the end the cuticle is founded to be stratified.

The present species appears to be most closely related in form and habit to *Tylencholaimus affinis* BRAKENHOFF, but may be distinguished from it by the following main points:—1) Lips absent; 2) oesophagus enlarged to form a bulb in the hindmost fifth; 3) vulva situated near the middle of the body; 4) male with an anal and 6–7 preanal papillae at varying intervals.



Text-fig. 35. *Dorylaimus brigdammensis* DE MAN. Anterior part of the female.

36. *Dorylaimus brigdammensis* DE MAN, 1876.

Text-fig. 35.

No=5 ♀ 2 Larvae 3

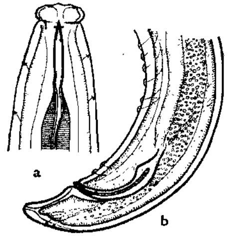
♀ L=1.83–2.12 mm. a=32.5–38.2

β=5.7.–6.5 γ=9.1–11.2

V=40.9–43.8%

Body slender, tapering slightly forwards and extremely backwards. Cuticle thick and naked. Anterior end truncated, with 6 slightly developed lips which bear small innervated papillae arranged

in 2 circles. Oral spear moderately strong. Oesophagus narrowed in the anterior half, rapidly swollen in the posterior. Female genital tube paired, symmetrical. Vulva situated in front of the middle of the body. Tail long, thread-like, 9–11 in the value of γ.



Text-fig. 36. *Dorylaimus carteri* BASTIAN.
a. Anterior part of the male.
b. Posterior part of the male.

37. *Dorylaimus carteri* BASTIAN, 1865.

Text-fig. 36.

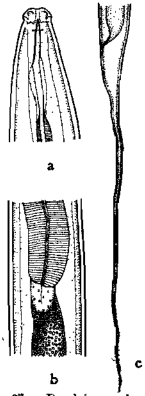
No=13 ♀ 5 ♂ 3 Larvae 5
(Sex. coef.=60 n=8)
♀ L=2.13–2.71 mm. α=30.1–38.7
β=4.2–4.9 γ=27.5–33.8
V=51.3–54.6%
♂ L=2.25–4.18 mm. α=36.3–44.0
β=4.1–4.5 γ=34.2–41.1
Pn=(1–2)+(11–14)

Body slender, tapering towards both ends. Cuticle thick and naked. Anterior end with 6 low distinct lips, each bearing 2 innervated papillae. Spear strong. Oesophagus gradually swollen in the posterior half. Female genital tube paired, symmetrical. Vulva lying a little behind the middle of the body. Male genital tube bifid. In a large male in hand there can be seen 2 anal papillae and a ventral row of 14 preanal papillae. Spicule plumpy, provided with a central thickening and a short accessory piece. Tail short, conoid but slightly pointed at the end, slightly curved ventrally in both sexes.

38. *Dorylaimus elegans* var. *longicaudatus* var. nov.

Text-fig. 37.

No=48 ♀ 6 Larvae 42
♀ L=2.72 (2.67–2.98) mm.
α=55.1(54.2–56.8) β=7.5(7.2–8.0)
γ=6.7(6.6–7.0)
V=38.3(37.1–40.1)%



Text-fig. 37. *Dorylaimus elegans* var *longicaudatus* var. nov.
a. Anterior part of the female.
b. Oesophagus-end region of the female.
c. Posterior part of the female.

Body very slender, tapering towards both ends, especially backwards. Anterior end naked, low disk-like shaped, slightly concave at the middle, without lip, but with a crown of 6 large innervated papillae. Oral spear moderately strong. Oesophagus extremely narrowed in the anterior two-thirds, gradually swollen in the posterior one-third. Female genital tube paired, symmetrical. Vulva situated near the hind end of the anterior two-fifths of the body. Tail extremely long, thread-like, ending in an extremely fine hair-like terminus. Male unknown.

This new variety may be distinguished from the original form by the possession of an extremely fine hair-like terminus.

39. *Dorylaimus filiformis* var. *papillatus* var. nov.

Text-fig. 38.

No=706 ♀ 64 ♂ 19 Larvae 623 (Sex. coef.=29.7 n=83)

♀ L=2.82 (2.28−3.27) mm.

α=42.5 (34.0−46.9) β=4.7 (4.1−5.3)

γ=9.2 (7.6−10.9)

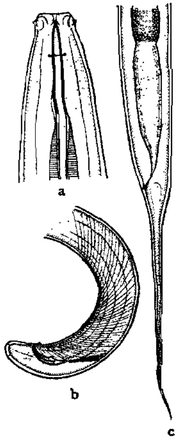
V=45.3 (42.6−49.5)%

♂ L=2.48 (2.27−3.88) mm.

α=42.2 (40.2−45.1) β=4.4 (4.2−4.7)

γ=80.1 (75.8−85.2)

Pn=1+30 (27−37)



Text-fig. 38. *Dorylaimus filiformis* var. *papillatus* var. nov.
a. Anterior part of the female.
b. Posterior part of the male.
c. Posterior part of the female.

Body very slender, tapering towards both ends, especially backwards in the female. Cuticle naked, unstriated. Anterior end truncated, without lip, bearing a crown of 6 innervated papillae. Oral spear rather strong. Oesophagus enlarged in the posterior half. Female genital tube paired, symmetrical. Vulva located slightly anterior to the middle of the body. Male genital tube bifid. Spicule rather slender, provided with a central thickening. Accessory piece indistinct. Male with an anal papilla and a median row of 27–37 ventral preanal papillae. Tail in the female gradually tapering towards the extremely fine hair-like terminus; in the male, shortly conoid,

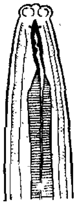
rounded and with 4 innervated papillae at the distal end, and slightly curved ventrally.

This new variety is characterized by the presence of ventro-median preanal papillae more numerous than in other varieties. This form was obtained in vast numbers corresponding to about 35% of the total number of individuals examined.

40. *Dorylaimus filiformis* var. *protumidus* var. nov.

Text-fig. 39.

No=298 ♀ 31 ♂ 15 Larvae 252
(Sex. coef.=48.4 n=46)
♀ L=1.53 (1.48−1.63) mm.
α=30.6 (29.6−32.6) β=4.7 (4.5−4.9)
γ=8.3 (7.5−9.3)
V=48.1 (45.9−52.0)%
♂ L=1.50 (1.41−1.72) mm.
α=32.1 (28.0−35.5) β=4.5 (4.2−5.0)
γ=60.1 (51.2−69.5) Pn=25 (23−27)



Text-fig. 39. *Dorylaimus filiformis* var. *protumidus* var. nov. Anterior part of the female.

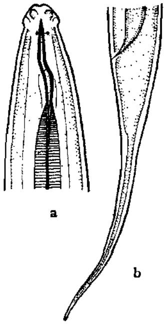
The present form, though most closely related to *D. filiformis* f. *longicaudatus* v. DADAY, differs from it as well as from other varieties by the following main points:— 1) Ventro-median preanal papillae more numerous than in others (23–27: 7–17); 2) some tooth-like chitinous protuberances are often found here and there in the inner wall of the spear.

41. *Dorylaimus limnophilus* DE MAN, 1880.

Text-fig. 40.

No=77 ♀ 23 Larvae 54
♀ L=0.98−1.21 mm. α=28.0−33.7 β=4.5−5.2 γ=7.4−9.3
V=32.7−36.3%

Body slender, tapering towards both ends, extremely posteriorly. Cuticle naked, unstriated. Anterior end somewhat triangular, concave at its extrem-



Text-fig. 40. *Dorylaimus limnophilus* DE MAN.
a. Anterior part of the female.
b. Posterior part of the female.

ity, without lip, but with a circle of 6 innervated papillae. Oral spear slender. Oesophagus swollen in the posterior one-fourth. Intestinal cells of a fairly deep yellowish colour. Female genital tube unpaired, postvulvar. Vulva situated near the hind end of the first third of the body. Tail elongate, gradually tapering to the pointed terminus. Male unknown.

42. *Dorylaimus micoletzkyi* DE MAN, 1921.

Text-fig. 41.

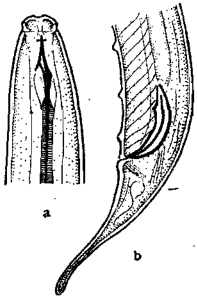
No=19 ♂ 3 Larvae 16

♂ L=1.57−1.62 mm. $\alpha=38.1-40.5$

$\beta=6.8-7.1$ $\gamma=20.2-22.4$

Pn=1+(6−7)

Body slender, tapering slightly anteriorly and extremely posteriorly. Cuticle unstriated, without papilla and bristle. Anterior end somewhat semispherical, slightly concave at the extremity, without lip, but bearing a crown of 6 innervated papillae. Oral spear slender, presenting near the base an aspect somewhat different from that in other species of the genus. Oesophagus narrowed anteriorly, rather abruptly swollen posteriorly from the beginning of the posterior one-third. Intestinal cells of a yellowish green colour. Male genital tube paired. Spicule rather slender, with a central thickening and an accessory piece. One anal papilla and 6–7 ventro-median preanal papillae present. Tail short, plumpy in the front, abruptly tapering to end in a bluntly pointed terminus. No specimen of the female came under my observation.



Text-fig. 41. *Dorylaimus micoletzkyi* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

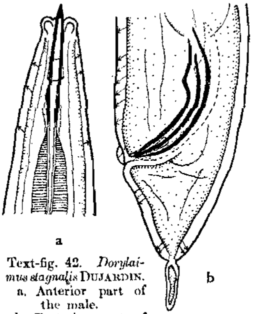
43. *Dorylaimus stagnalis* DUJARDIN, 1845.

Text-fig. 42.

No=7 ♀ 2 ♂ 1 Larvae 4 (Sex. coef.=50 n=3)

♀ L=3.71–3.82 mm. α=34.7–36.9. β=4.6–4.8 γ=12.6–13.5 V=45.3–46.7%

♂ L=2.61 mm. α=37.3 β=3.7 γ=37.1



Text-fig. 42. *Dorylaimus stagnalis* DUJARDIN.
a. Anterior part of the male.
b. Posterior part of the male.

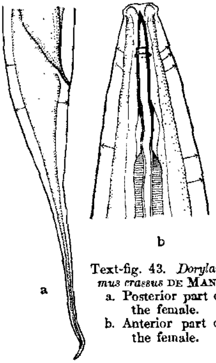
Body slender, slightly tapering forwards. Cuticle thick, unstriated, with innervated papillae. Anterior end not truncated, with 6 slightly developed lips, which bear 2 circles of 6 small innervated papillae. Oral spear strong. Guiding ring for the spear long, trapezoid. Oesophagus narrowed in the distal half, but gradually swollen backwards. Female genital tube paired, symmetrical. Vulva always situated in front of the middle of the body. Male genital tube bifid. Spicule plumpy, provided with a short accessory piece. One anal papilla and numerous innervated preanal papillae present. Tail long, thread-like in the female, but short, stumpy and somewhat rounded in the male.

44. *Dorylaimus crassus* DE MAN, 1880.

Text-fig. 43.

No=1 ♀ 1

♀ L=4.86 mm. α=29.8 β=4.8 γ=14.6 V=47.0%



Text-fig. 43. *Dorylaimus crassus* DE MAN.
a. Posterior part of the female.
b. Anterior part of the female.

Body fairly slender, considerably tapering forwards. Cuticle thick, unstriated, with innervated papillae. Anterior end nearly truncated, with 6 low indistinct lips which have 2 crowns of 6 small innervated papillae. Oral spear strong, its guiding ring

low, trapezoid. Oesophagus enlarged in the posterior half. Female genital tube paired, symmetrical. Tail long, thread-like, ending in an extremely fine hair-like terminus.

45. *Dorylaimus tambo* sp. nov.

Text-fig. 44.

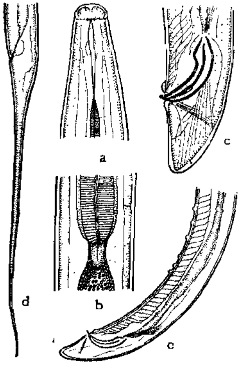
No=16 ♀ 2 ♂ 1 Larvae 13 (Sex. coef.=50 n=3)

♀ L=3.90 (3.58−4.30) mm. α=75.5 (74.6−76.6)

β=15.5 (14.3−17.2) γ=10.4 (10.2−10.8)

V=38.0 (37.6−38.5)%

♂ L=3.51 mm. α=66.0 β=14.1 γ=87.5 Pn=1+6



Text-fig. 44. *Dorylaimus tambo* sp. nov.
a. Anterior part of the male.
b. Oesophagus-end region of the male.
c. Anal region of the male.
d. Posterior part of the female.
e. Posterior part of the male.

Body extremely slender, slightly tapering forwards. Cuticle thin, transparent, colourless, unstriated, and naked. Anterior end almost rounded, without lip, but with 2 crowns of 6 innervated papillae. Just behind the mouth occurs a ring-shaped thickening of chitinous intima for the spear, which is short and slender. Oesophagus very short (♀ β=15.5, ♂ β=14.1), enlarged in the posterior one-third. Cardia well developed, almost cylindroid, disposed from the end of the oesophagus into the intestine. Intestine distinctly separated from the oesophagus by a constriction, but soon becoming about three-fourths as wide as the body. Female genital tube unpaired, postvulvar. Vulva placed slightly in front of the third fifth of the body. Male genital tube bifid. Spicule rather slender, presenting a thickening at the center. Accessory piece present, one-third as long as the spicule. Male with one anal papilla and 6 preanal papillae in the mid-ventral line. Tail different in form and size in both sexes. In the female, it is very long and tapers rather regularly to the extremely fine hair-like terminus. In the male, however, it is very short, plumpy

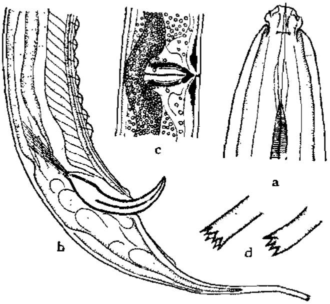
and conical with a rounded terminus which is slightly curved ventrally and bears 2 dorsal papillae near the end.

This new species is very much like *Dorylaimus tenuis* DITLEVSON, but may be clearly distinguished from it by the following main points:—1) Body shorter than in *D. tenuis* (♀ 3.9 mm., ♂ 3.5 mm. : ♀ 6 mm., ♂ 4.5 mm.); 2) tail highly different in form and size in both sexes; 3) anal and preanal papillae smaller in number (7 : 19); 4) an accessory piece is present in the male at the base of the spicule. A large number of specimens were found after irrigation, especially around the root of the rice plant.

46. *Dorylaimus denticaudatus* sp. nov.

Text-fig. 45.

No=244 ♀ 61 ♂ 21 Larvae 162 (Sex. coef.=34.4 n=82)
♀ L=3.06 (2.72–3.65) mm. α=60.1 (56.2–65.0) β=6.7 (6.0–7.1)
γ=18.6 (13.9–20.9) V=46.5 (43.1–52.3)%
♂ L=2.72 (2.50–2.85) mm. α=59.4 (58.1–61.1) β=6.4 (5.6–6.8)
γ=21.7 (20.8–23.1) Pn=1+14 (13–17)



Text-fig. 45. *Dorylaimus denticaudatus* sp. nov.
a. Dorso-median view of the anterior end.
b. Posterior part of the male.
c. Vulvar region of the female.
d. Two types of the terminus.

Body extremely slender, tapering slightly anteriorly and extremely posteriorly. Cuticle moderately thick, transparent, colourless, unstriated, and naked. Anterior end almost semispherical, somewhat expanded at the extremity, without lip, but with 2 crowns of 6 small innervated papillae. Spear very slender, its guiding ring present near the mouth. Oesophagus very narrow in the anterior two-fifths, thence suddenly thickened backwards to about two-thirds the breadth of the body at the corresponding level. In-

testine narrow, not exceeding the maximum width of the oesophagus. Prerectum distinct, about 11–14 times as long as the breadth of the body at the corresponding level. Female genital tube paired, symmetrical. Vulva located slightly in front of the middle of the body. Male genital tube paired. Spicule rather slender, with a central thickening which attains about two thirds the length of the spicule. No accessory piece present. Male with an anal papilla and a median row of 13–17 ventral preanal papillae. Just behind the anus exists a peculiar postanal ventro-median cuticular rib, which is about as long as the central thickening of the spicule. Tail almost identical in form and size in both sexes, tapering rather regularly from the anus to the narrow tube-like terminus, ending in 4 or 6 sharp points in circle as in the case of *Spiroptera pulicis* v. LINSTOW.

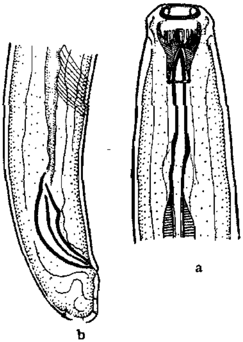
Numerous individuals of this new species were obtained before irrigation. Occasionally I could find some individuals which were infested with a certain fungus on the body surface, especially at the end of the tail.

47. *Actinolaimus macrolaimus* DE MAN, 1880.

Text-fig. 46.

No = 17 ♂ 1 Larvae 16

♂ L = 2.40 mm. $\alpha = 43.6$ $\beta = 4.1$ $\gamma = 96.0$



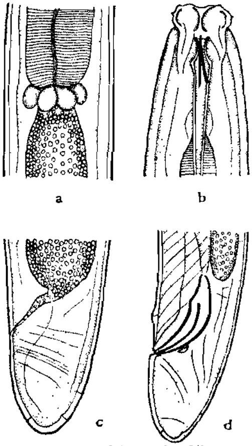
Text-fig. 46. *Actinolaimus macrolaimus* DE MAN.
a. Anterior part of the male.
b. Posterior part of the male.

Body slender, slightly tapering towards both ends in the male. Cuticle naked, unstriated. Lateral organ indistinct. Anterior end truncated, disk-like shaped, without lip, but with a crown of 6 innervated papillae. Vestiblum typical, with apparent chitinous stripes. Spear moderately strong. Oesophagus swollen in the posterior half. Male genital tube paired. Spicule fairly plumpy, with a central thickening and an accessory piece. Tail short and blunt. Male with many ventro-median preanal papillae arranged in two groups. No female specimen came under my observation.

48. *Nygolaimus kaburakii* sp. nov.

Text-fig. 47.

No=244 ♀ 23 ♂ 19 Larvae 182 (Sex. coef.=82.6 n=42)
♀ L=1.78 (1.51–1.93) mm. a=34.0 (30.2–37.5) β=3.9 (3.6–4.3) γ=62.3 (52.6–74.8) V=52.2 (49.5–55.1)%
♂ L=1.72 (1.62–1.82) mm. a=33.7 (31.0–36.2) β=3.8 (3.4–4.1) γ=51.2 (45.3–55.1) Pn=1+(2–5)



Text-fig. 47. *Nygolaimus kaburakii* sp. nov.
a. Oesophagus-end region of the male.
b. Dorso-median view of the anterior end.
c. Anal region of the female.
d. Anal region of the male.

Body slender, slightly tapering towards both ends. Cuticle moderately thick, transparent, colourless, traversed by extremely fine transverse striae which are easily visible in the head and tail. Lateral organ linear, not so large, distinct only at dorso-median aspect. Anterior end more or less conoid at dorsal aspect, slightly concave at the extremity, but cylindroid posteriorly, with 6 lips, each bearing 2 innervated papillae. Pharynx narrowed anteriorly, round-pen-shaped either at dorsal or at lateral view, with a fairly long ventral submedian spear. Spear longer as compared with that of the other species of this genus, measuring 13.5μ in one individual which is 1.75 mm. long. Occasionally the spear was observed to thrust in and out from the mouth under the microscope. Oesophagus dorylaimoid, enlarged in the posterior half, about two-thirds as wide as the body at the level of its base. At the proximal end of the oesophagus is found a gland consisting of 6 lobes, instead of the ventral gland (Text-fig. 47a). Intestinal cells of a deep yellowish colour. Prerectum distinct, three times as long as the width of the body at the corresponding level. Female genital tube paired, symmetrical. Vulva situated near the middle of the body. Male genital tube paired. Spicule fairly slender, thickened at the center. Accessory piece present near the disal end of the spicule, very small, tri-

angular as is seen in some species of *Mononchus*. Male with one anal papilla and 2–5 low preanal papillae at varying intervals. Tail short, plumpy, almost semispherical, with 6 innervated papillae posteriorly, of which 2 are dorsal and 4 ventral. Tail somewhat longer in the male than in the female.

This new species seems to be closely related to *N. denticulatus* COBB and *N. menzeli* MICOLETZKY, but differs from them by the following main points :—1) Body shorter than in these two species ; 2) male very frequently visible ; 3) pharynx narrowed anteriorly, round-pen-shaped either at dorsal or at lateral view ; 4) spear longer than that of the two species ; 5) oesophageal gland occurs at the proximal end of the oesophagus which consists of 6 lobes ; 6) prerectum moderately long, three times as long as the width of the body at the corresponding level, longer than that of *N. menzeli*, but shorter than that of *N. denticulatus*.

Notes on the Nemic Population before and after Irrigation.

In order to observe changes in the nemic population before and after irrigation the number of individuals occurring in each of five soil layers was counted. The results obtained are given in Table 1.

TABLE I.

Numbers of free-living nemas found in the paddy field before and after irrigation.

Specific name \ Layer	Before irrigation							After irrigation							
	I	II	III	IV	V	Total	%	I	II	III	IV	V	Total	%	Sum
**Actinolaimus macrolaimus*	11	1	0	0	0	12	70.6	3	0	0	0	2	5	29.4	17
Alaimus lemani	3	0	0	0	1	4	3.4	32	47	26	8	1	114	96.6	118
—— *primitivus*	36	1	2	0	0	39	21.8	48	36	25	23	8	140	78.2	179
**Aphelenchus bicaudatus*	0	0	4	0	0	4	22.2	7	0	7	0	0	14	77.8	18
Bastiana exilis	1	0	0	0	0	1	7.1	3	8	0	2	0	13	92.9	14
Cephalobus elongatus	1	1	0	1	0	3	75.0	1	0	0	0	0	1	25.0	4
—— *emarginatus*	0	0	0	0	0	0	0	0	0	0	1	0	1	100.0	1

TABLE I. (Continued).

Specific name \ Layer	Before irrigation I	II	III	IV	V	Total	%	After irrigation I	II	III	IV	V	Total	%	Sum
Cephalobus persegnis	0	0	1	1	0	2	100.0	0	0	0	0	0	0	0	2
Chriptonchus nudus	6	0	0	0	0	6	75.0	0	2	0	0	0	2	25.0	8
Chromadola fluviatilis	0	0	0	0	0	0	0	1	0	0	0	0	1	100.0	1
Chronogaster gracilis	2	2	0	0	0	4	44.4	1	4	0	0	0	5	55.6	9
**Criconema komabaensis*	0	0	0	0	0	0	0	0	2	0	0	0	2	100.0	2
Cyatholaimus ruricola	0	0	0	0	0	0	0	2	4	3	0	0	9	100.0	9
**Dorylaimus brigdammensis*	0	0	0	0	0	0	0	2	3	0	0	0	5	100.0	5
* —— *carteri*	5	3	1	1	0	10	76.9	0	2	1	0	0	3	23.1	13
* —— *crassus*	0	0	1	0	0	1	100.0	0	0	0	0	0	0	0	1
* —— *denticaudatus*	3	198	41	0	0	242	99.2	2	0	0	0	0	2	0.8	244
* —— *elegans* var. *longicaudatus*	0	0	0	0	0	0	0	0	3	35	8	2	48	100.0	48
* —— *filiformis* var. *papillatus*	293	251	42	12	6	604	85.5	27	36	26	9	4	102	14.5	706
* —— *filiformis* var. *protumidus*	6	207	55	22	2	292	98.0	2	4	0	0	0	6	2.0	298
* —— *limnophilus*	1	0	0	0	0	1	1.3	2	23	30	11	10	76	98.7	77
* —— *micoletzkyi*	2	0	0	0	0	2	10.5	0	11	4	0	2	17	89.5	19
* —— *stagnalis*	0	0	7	0	0	7	100.0	0	0	0	0	0	0	0	7
* —— *tambo*	0	0	0	0	0	0	0	1	0	0	13	2	16	100.0	16
Ironus ignavus	63	1	0	1	0	65	67.7	23	8	0	0	0	31	32.3	96

TABLE I. (Continued).

Specific name \ Layer	Before irrigation							After irrigation							Sum
	I	II	III	IV	V	Total	%	I	II	III	IV	V	Total	%	
Monhystera agilis	1	0	0	0	0	1	100.0	0	0	0	0	0	0	0	1
——— *filiformis*	0	2	0	0	0	2	14.3	7	5	2	0	0	14	85.7	16
——— *paludicola*	0	0	0	0	0	0	0	1	0	0	0	0	1	100.0	1
Mononchus macrostoma	2	0	1	0	2	5	9.8	4	5	28	6	3	46	90.2	51
——— *tunbrigdensis*	2	1	10	4	0	17	36.9	8	4	6	9	2	29	63.1	46
**Nygolaimus kaburakii*	120	6	11	1	1	139	62.1	22	25	19	6	13	85	37.9	224
Oncholaimus punctatus	1	0	0	0	0	1	100.0	0	0	0	0	0	0	0	1
Plectus cirratus	3	1	16	0	1	21	75.0	0	0	4	3	0	7	25.0	28
——— *multitubiferus*	2	0	0	0	0	2	40.0	0	3	0	0	0	3	60.0	5
Prismatolaimus dolichulus	1	0	1	0	6	2	66.7	1	0	0	0	0	1	33.3	3
Pseudoaulolaimus anchilocaudatus	4	0	2	2	0	8	7.2	25	30	36	9	3	103	92.8	111
Rhabdolaimus aquaticus	5	0	0	0	0	5	62.5	1	0	0	2	0	3	37.5	8
Trilobus gracilis	0	0	1	0	0	1	50.0	1	0	0	0	0	1	50.0	2
Tripyla papillata	21	2	4	0	0	27	58.7	6	0	7	6	0	19	41.3	46
**Tylencholaimus bryophilus*	0	0	0	0	0	0	0	5	32	7	2	2	48	100.0	48
* ——— *longicaudatus*	0	0	0	0	0	0	0	2	14	2	4	7	29	100.0	29
* ——— *mirabilis*	0	0	0	0	0	0	0	0	1	0	0	1	2	100.0	2
**Tylenchus apapillatus*	0	2	31	14	4	51	91.1	0	0	2	0	3	5	8.9	56

TABLE I. (Continued).

Specific name \ Layer	Before irrigation							After irrigation							Sum
	I	II	III	IV	V	Total	%	I	II	III	IV	V	Total	%	Sum
**Tylenchus filiformis*	0	0	7	1	1	9	31.0	4	0	0	0	16	20	69.0	29
* —— *gracilis*	11	190	118	100	38	457	95.6	0	1	0	4	16	21	4.4	478
* —— *intermedius*	0	0	5	0	0	5	21.7	5	7	5	1	0	18	78.3	23
* —— *leptosoma*	0	0	1	0	0	1	50.0	1	0	0	0	0	1	50.0	2
Walcherenia typica	0	0	0	0	0	0	0	0	1	0	0	0	1	100.0	1
Sum	606	869	362	160	56	(2053)	65.7	250	321	275	127	97	(1070)	34.3	(3123)
Total number of species belonging to Anguillulinidae	452	858	324	151	52	(1837)	77.7	86	164	138	58	80	(526)	22.3	(2363)

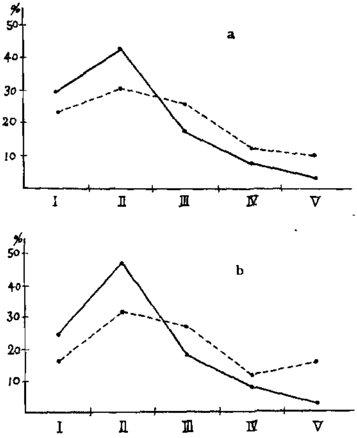
As is evident from the table, the number of individuals amounts in all to 2,053 in the field before irrigation (the first plot), and 1,070 after irrigation (the second plot). Accordingly the nemic population after irrigation is said to be reduced to about half what it was before irrigation. This reduction after irrigation appears to me to be induced by the drowning and removal to the roots of the young rice plants or elsewhere.

Now let us proceed to obtain some insight upon the changes in number before and after irrigation of each species which amounts to more than ten. From a consideration of the results given in Table 1, the following species, though different in degree, appear to present a positive tendency to the irrigation water: *Alaimus lemani*, *A. primitivus*, *Bastiana exilis*, *Dorylaimus elegans* var. *longicaudatus*, *D. limnophilus*, *D. micoletzkyi*, *D. tambo*, *Monhystera filiformis*, *Mononchus macrostoma*, *Tylencholaimus bryophilus*, *T. longicaudatus*. On the other hand, the following appear to represent the species having the negative tendency to the water: *Dorylaimus denticaudatus*, *D. filiformis* var. *papillatus*, *D. filiformis* var. *protumidus*, *Ironus ignavus*, *Nygolaimus kaburakii*, *Tylenchus apapillatus*, *T. gracilis*.

TABLE II.

Percentage of free-living nemas and the Anguillulinidae in the paddy field before and after irrigation.

	Before irrigation					After irrigation				
Layer	I	II	III	IV	V	I	II	III	IV	V
Free-living nemas	29.51	42.33	17.63	7.80	2.73	23.36	30.00	25.70	11.87	9.07
Anguillulinidae	24.60	46.70	17.63	8.22	2.85	16.35	31.18	26.23	11.03	15.21



Text-fig. 48. a. Graphs showing the depth distribution of free-living nemas in the paddy field before (full line) and after (broken line) irrigation.

b. Graphs showing the depth distribution of Anguillulinidae in the paddy field before (full line) and after (broken line) irrigation.

So far as examinations are concerned, the vertical distributions of the nemas before and after irrigation are quite similar in state, the maximum being attained at the second layer where the root system of the rice plant occurs, as in Table 2 and Text-fig. 48a.

The nemic population after irrigation, although thus assuming a state similar in distribution to that before irrigation, is found to undergo a marked decline in the first and second layers more than in the others. This change is in all probability due to the fact that the population in the upper soil mass has suffered from some marked disturbances within the scope of ploughing.

Now glancing at the number of species belonging to the Anguillulinidae (indicated by asterisk in Table 1) which are considered to be more or less noxious to the higher plants, they number 1,837 before irrigation and 526 after irrigation, or 2,363 in all. This total number corresponds to 75.6% of the entire nemas obtained from the two plots. Their depth distribution is as in Table 2 and Text-fig. 48b. Comparing the graphs with those given in Text-fig. 48a, it may be evident that the distributional state of the nemas as a whole before irrigation is determined by the population of the Anguillulinidae, but this is not true of the case after irrigation. After irrigation the distributional state of the nemas as a whole appears to be considerably determined by the population of the so-called fresh-water nemas, such as *Alaimus*, *Bastiana*, *Monhystera*, *Mononchus*, *Pseudoaulolaimus*, etc. Notwithstanding the reduction of the Anguillulinidae to less than one-third, the predatory *Mononchus* were found to increase to three times and a half as much as that before irrigation. This appears to suggest that the predatory nemas are effective to a certain extent in checking the nemas injurious to the young rice plants in the paddy field at the beginning of the irrigation season.

Summary.

The present investigation was undertaken to determine the free-living nematodes in the paddy field of the University farm at Komaba near Tokyo and some changes of their population before and after irrigation.

1) The number of individuals examined amounted to 3,123 in all, which were referable to 25 genera and 48 forms.

2) The nemic population after irrigation was reduced as a whole to about half as much as that before irrigation. As regards each species, how-

ever, the changes of the population before and after irrigation differ according to different species.

3) The nemic population after irrigation presented a marked decline in the upper soil mass, probably due to ploughing.

4) Before and after irrigation the maximum nemic population was encountered at the second layer, where the root system of the rice plant occurs.

5) The number of species belonging to the Anguillulinidae amounted to 2,363 in all, this corresponding to 75.6% of the entire nemas obtained from the two plots. After irrigation these nemas were reduced to less than one-third as much as that before irrigation. This appears to suggest that the irrigation water is highly effective for checking the nemas injurious to the young rice plants either by drowning them or by inducing the propagation of such a predatory form as *Mononchus*.

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The Genetics of Flower Colours in Pharbitis Nil.

By

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With Plate XVI.

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Introduction.

The Japanese morning glory (*Pharbitis Nil*) is a favorable material for the genetic study, for the reason that it includes many distinguishable characters. In regard to the flower colour of this plant a number of genetic papers have been published by some Japanese investigators. Sô (1915) described the genetics of purple, red and white flowers, without numerical data. TAKEZAKI (1916) first detected two complemental genes for the production of the flower colour, by crossing two whites. MIYAZAWA (1918, 1921), crossing his so-called dark red (dusky) with white, obtained results showing the recessive nature of white to coloured, dark red to normal (blue) and red to purple. Afterwards, IMAI (1919) studied the recessive nature of dusky to normal; and in other papers (1921 a,b), he reported in more detail on the complemental genes studied by TAKEZAKI. MIYAKE and IMAI (1920) described the relation between light and dark colours. The writer (HAGIWARA 1923) showed the relation of blue, purple (including

magenta), red and dark red (dusky), giving three genes: purple, magenta and dusky. Afterwards the writer extended the study, partly proving his previous results, and identified the corresponding variation in the broken (dusky) colour series (1928a). He (1928b) found another new broken colour (duskish), which is due to a gene, not allelomorphic to dusky mentioned above. Furthermore, he (1928a, 1929a,b, 1930) detected a gene, the dominant allelomorph of which is complementary in the production of the coloured flower with the two, previously detected genes. Recently, IMAI (1930) published a paper, in which he gave the confirmation of the facts reported hitherto on the interrelations of the normal four colours, dusky, duskish and whites.

In this paper, the writer will describe the interrelation of the various flower colours in detail.

Flower Colour Series.

The flower colour series includes blue, purple, magenta, red, slate, gray, plum, drab, terra-cotta, vinaceous, and also light yellow and white. Depending on the fact that we find only the description of light blue in old Chinese and Japanese books, we may regard the light blue flower as the origiual form of this plant.

The general flower colours exhibited by anthocyanin pigments may be gathered under the two groups, pure and broken colours, the former containing blue, purple, magenta and red, and the latter including slate, gray, plum, drab, terra-cotta and vinaceous. In the following lines, the respective colours are indicated by the directions according to Ridgway's colour standards.

Group	Colour		Ridgway's	Colour No.
Pure Colours	Blue		nearly	45
	Purple		”	55–59
	Magenta		”	61–63
	Red		”	65–69
Broken Colours I	Slate		”	$49'''$–$57'''$
	Plum		”	$61'''$–$65'''$
	Terra-cotta	I	”	$1'''$–$9'''$
		II	”	$7''$
Broken Colours II	Gray		”	$57''''$
	Drab		”	$1'''$
	Vinaceous	I	”	$1''$
		II	”	$1''$

The other group contains white and light yellow, in which no anthocyanin pigment is developed. Such flowers can be classified into several forms by the colours of the flower tube, stem and seed; namely, white with coloured tube (Fig. 9), white with coloured stem (Fig. 11), white with white tube (Fig. 10), white bearing white seed, and creamish (Fig. 12). In the present paper the writer, however, will reserve the description of the data on this group which will be reported in the future.

In Tokyo the seeds of this plant are generally sown in early May and its flowers bloom from July to October. Corollas are very thin in texture and their colour changes by sunshine. Such colours as blue and purple are liable to change into reddish. Therefore, the observation on flower colours must be made very early in the morning.

Experiments.

The experiments, the results of which will be described here, concern the twelve flower colour phenotypes of the pure colours and the broken colours, disregarding the other points, such as the intensity, pattern etc.

I. Pure Colours.

The genetic interrelations among four colour groups—blue, purple, magenta and red—were brought to light by the data involving twenty-five crosses, the results of which are presented in the following six sections.

Purple versus blue. Six different crosses were made between purple and blue flowers, giving F_1 plants which bore blue flowers. In F_2, purple flowers reappeared in one-fourth of the total, as shown in Table I.

Table I.

F_2 plants from the cross of purple and blue.

Cross	Blue	Purple	Total
41×54	63	22	85
56×57	40	13	53
135×118	39	11	50
92.3×100.2	71	27	98
431.1×419.51	49	15	64
419.51×A	40	11	51
Total	302	99	401
Expected	300.75	100.25	401

Blue behaves as a dominant character to purple, and a recessive gene p_r (previously b) is responsible for purple.

MAGENTA VERSUS BLUE. Crossing a blue flowered plant (No. 85.1) with a magenta flowered plant (No. 70.2) F_1 plants bearing blue flowers were obtained. In F_2, 15 blue and 4 magenta were recorded, showing that blue is a simple dominant character to magenta, though the numbers observed were very few. An F_3 obtained by selfing blue F_2 plants confirms this point, as shown by the data in Table II.

TABLE II.

F_3 of cross 85.1 × 70.2.

F_2 flower	No. families	Blue	Magenta	Total
Blue	5	83	—	83
	9	166	68	234

Therefore, magenta differs by one gene from the normal. Let us designate the gene by m_g (previously p.)

RED VERSUS PURPLE. From the experiments above shown, we learned the fact that purple and magenta are simple recessives to blue. In the present section let us describe the results in their relation to the inheritance of red.

The F_1 plants obtained by the cross between red and purple flowers bore purple corolla; and in F_2, a simple segregation occurred, giving purple and red flowers in the ratio 3 : 1, as shown in Table III.

TABLE III.

F_2 plants from the cross of red and purple.

Cross	Purple	Red	Total
72×41	52	13	65
56×72	100	41	141
468.18×499.47	38	9	47
A×R.1	35	11	46
56×V-Y	46	16	62
Total	271	90	361
Expected	270.75	90.25	361

The further generation of cross 468.18 × 499.47 was observed, giving expected results as shown in Table IV.

TABLE IV.

F_3 of cross 468.18 × 499.47.

F_2 flower	No. families	Purple	Red	Total
Purple	11	99	—	99
	27	369	125	494
Red	7	—	70	70

Hence, red is a simple recessive to purple in inheritance. From the fact that the F_1 bore purple flowers but not reversional blue flowers, we may conclude that the red flower cannot contain the dominant allelomorph for purple.

RED VERSUS MAGENTA. The writer made three crosses between red and magenta, and obtained purple flowers as F_1. In F_2, magenta hybrids gave magenta and red segregates in a monohybrid ratio, indicating that red is recessive to magenta. The numerical data are shown in Table V.

TABLE V.

F_2 plants from the cross of red and magenta.

Cross	Magenta	Red	Total
60 × 72	27	10	37
103.3 × 60	30	8	38
129.3 × 133	28	9	37
Total	85	27	112
Expected	*84.0*	*28.0*	112

Therefore, the red flower cannot include either P_r nor M_g in its gene complex.

RED VERSUS BLUE. The cross between blue and red is expected to give a key to understanding interrelation among four groups of the pure flower colours. The F_1 plants bore blue flowers; and in F_2, the segregation occurred into blue, purple, magenta and red, as shown by the data in Table VI.

TABLE VI.

F_2 plants from the cross of red and blue.

Cross	Blue	Purple	Magenta	Red	Total
72×53	61	23	25	13	122
85.1×103.6	25	6	6	5	42
130.2×100.3	25	5	4	3	37
100.3×103.7	22	7	5	6	40
127×110	20	5	6	1	32
233.36×457.15	27	8	8	5	48
159×V	49	10	13	10	82
233.36×428.15	33	9	10	5	57
Total	262	73	77	48	460
Expected	258.75	86.25	86.25	28.75	460

$\chi^2 = 15.87$ $P = 0.001$

The segregation occurred with a remarkable deviation, but it does not deny the assortment of two genes, purple and magenta. Red is double recessive, that is $p_r\ m_y$. The further generation of 53 F_2 plants, obtained by the cross 233.36×428.15, was observed, giving the results as shown in Table VII.

TABLE VII.

F_3 of cross 233.36×428.15.

F_2 flower	No. families	Blue	Purple	Magenta	Red	Total
Blue	3	34	—	—	—	34
	10	69	—	28	—	97

F_2 flower	No. families	Blue	Purple	Magenta	Red	Total
Blue	10	112	35	—	—	147
	9	82	29	29	16	156
Purple	3	—	53	—	—	53
	4	—	76	—	23	99
Magenta	5	—	—	50	—	50
	5	—	—	45	13	58
Red	5	—	—	—	112	112

The F_3 data cover the theory proposed above, namely, red is double recessive to blue.

MAGENTA VERSUS PURPLE. Magenta and purple being recessive to blue, what result will be obtained by crossing the two recessives? The cross gave reversional blue flowers as F_1; and in F_2, segregation occurred as indicated by the data in Table VIII.

TABLE VIII.

F_2 plants from the cross of magenta and purple.

Cross	*Blue*	*Purple*	*Magenta*	*Red*	*Total*
56×60	189	53	60	25	327
468.18×499.49	19	5	6	1	31
499.25×499.9	34	10	10	2	56
499.25×499.17	28	8	10	3	49
409.16×415.32	60	27	28	4	119
415.43×454.2	40	16	17	5	78
Total	370	119	131	40	660
Expected	371.25	123.75	123.75	41.25	660

$\chi^2=0.64$ $\quad$ $P \doteq 1$

The segregation occurred in accordance with a dihybrid ratio. Since the genotype of F_1 is $P_rp_rM_gm_g$, the F_2 segregation should be 9 blue, 3 purple, 3 magenta and 1 red in every 16. The F_3 generation of 61 F_2 plants from the cross 415.43 × 454.2 was raised, and the data obtained are shown in Table IX.

TABLE IX.

F_3 of cross 415.43 × 454.2.

F_2 flower	No. families	Blue	Purple	Magenta	Red	Total
Blue	4	63	—	—	—	63
	8	64	—	24	—	88
	10	137	33	—	—	170
	17	289	78	86	28	481
Purple	3	—	32	—	—	32
	3	—	35	—	10	45
Magenta	6	—	—	65	—	65
	6	—	—	109	33	142
Red	4	—	—	—	79	79

The results perfectly cover the expectation developed on the basis of dihybrid.

From the experimental data shown in the foregoing pages the genotypes of the four pure flower colours are considered as follows:

Blue .... P_rM_g
Purple .... p_rM_g
Magenta .... P_rm_g
Red .... p_rm_g

So far as the writer's experiments went, no exceptional cases beyond the assumption were found.

II. Broken Colours.

The broken colours have a dull hue, contrasting to pure colours, which have a bright hue. As mentioned before, the broken colours are classified into two groups—broken I and broken II. In both broken colour series, there occur variations corresponding to the pure colour series.

In connection with the broken colours, 39 crosses were made, the results being described in the following two parts.

Broken colour I.

The dull colours, corresponding to magenta and red, are difficult to detect in some cases. Therefore, the writer gathered them into a terra-cotta group, though they were classified into terra-cotta I and terra-cotta II in other cases.

Slate versus blue. Four crosses were made between slate and blue. All F_1 plants bore blue flowers; and in F_2, blue and slate were segregated after a monohybrid fashion, the data obtained being shown in Table X.

Table X.

F_2 plants from the cross of slate and blue.

Cross	Blue	Slate	Total
44×37.4	43	10	53
124.2×100.23	30	8	38
141×X	49	13	62
233.36×455.22	36	13	49
Total	158	44	202
Expected	151.5	50.5	202

Slate is, therefore, a simple recessive to blue, and the character is due to a gene dusky or d_y (formerly k^1). Consequently slate is considered to carry a genetic complex $P_rM_gd_y$.

Slate versus purple. The F_2 plants obtained by two crosses between

slate and purple bore blue flowers, differing from both parents. In F_2 segregation took place dihybridly, as shown in Table XI.

TABLE XI.

F_2 plants from the cross of slate and purple.

Cross	Blue	Purple	Slate	Plum	Total
58 × 41	53	21	11	3	88
51A × 56	43	19	16	4	82
615.58×499.26	16	9	9	1	35
Total	112	49	36	8	205
Expected	115.31	38.44	38.44	12.81	205

$\chi^2=4.96$ P=0.18

From the double heterozygous constitution of the F_1 blue flowers we should expect 9 blue, 3 purple, 3 slate, and 1 plum in every 16 F_2. The expectations in the above table were calculated from such a ratio; therefore, plum is double recessive, that is $p_rM_gd_y$.

PLUM VERSUS SLATE. The F_1 hybrids obtained by crossing plum with slate bore slate flowers, and they segregated into slate and plum in a ratio 3: 1, as shown by the data in Table XII.

TABLE XII.

F_2 plants from the cross of plum and slate.

Cross	Slate	Plum	Total
141×41/C.1	87	35	122
Expected	91.5	30.5	122

The result indicated in the table confirms the fact that the genotype of plum is purple dusky or $p_rM_gd_y$. The F_3 generation was raised, and the data are gathered in Table XIII.

TABLE XIII.

F_3 of cross 141×41/C.1.

F_2 flower	No. families	Slate	Plum	Total
Slate	8	97	—	97
	28	298	86	384
Plum	12	—	162	162

The results confirm the simple recessive nature of plum to slate.

PLUM VERSUS PURPLE. An F_1 obtained by crossing plum (No. 419.17) with purple (No. 407.24) bore purple flowers; and in F_2, 33 purple and 12 plum were segregated, giving a 3 : 1 ratio as expected.

PLUM VERSUS BLUE. Crossing blue (No. 233.36) with plum (No. 41/C.1), blue flowers were produced as F_1. In F_2, 11 blue, 5 purple, 3 slate and 2 plum were segregated, according to an expected dihybrid ratio. Though the data are small, they may confirm, together with the results given in previous sections, the double recessive nature of plum.

SLATE VERSUS MAGENTA. A cross was made between slate and magenta, and reversional blue-flowered F_1 plants were obtained. In F_2, segregation occurred as shown in Table XIV.

TABLE XIV.

F_2 plants from the cross of slate and magenta.

Cross	Blue	Magenta	Slate	Terra-cotta	Total
37.4×70.2	27	7	9	1	44
530.10×516.41	75	24	17	6	122
Total	102	31	26	7	166
Expected	93.4	31.1	31.1	10.4	166

$\chi^2=2.74$ $P=0.57$

The parental constitutions in this cross are considered to be $P_r M_g d_y$ for slate and $P_r m_g D_y$ for magenta. Therefore, dihybrid segregation may be expected in F_2, the assortment being concerned with the two genes, m_g and d_y. The segregated terra-cotta flowers correspond to the double recessives. The theory will be proved by the F_3 data, which are presented in Table XV.

TABLE XV.

F_3 of cross 37.4 × 70.2.

F_2 flower	No. families	Blue	Magenta	Slate	Terra-cotta	Total
Blue	2	31	—	—	—	31
	5	67	—	25	—	92
	7	72	33	—	—	105
	13	141	38	42	15	236
Magenta	3	—	23	—	—	23
	3	—	37	—	15	52
Slate	3	—	—	36	—	36
	3	—	—	58	20	78
Terra-cotta	1	—	—	—	22	22

Inspecting the data shown in the above table, the double recessive nature ($P_r m_g d_y$) of terra-cotta is clear.

TERRA-COTTA I VERSUS BLUE. The F_1 hybrids obtained by crossing terra-cotta with blue bore blue flowers, and they segregated into four forms in F_2 as shown by the data in Table XVI.

TABLE XVI.

F_2 plants from the cross of terra-cotta I and blue.

Cross	Blue	Magenta	Slate	Terra-cotta I	Total
233.1 × 416.37	*39*	*20*	*21*	*6*	*86*
Expected	48.4	16.1	16.1	5.4	86

$\chi^2 = 4.33$ P=0.23

The result is practically the same with that shown in Table XIV.

TERRA-COTTA II VERSUS PLUM. Two crosses were made between plum and terra-cotta II. The F_1 plants thus obtained bore plum flowers. In F_2, segregation occurred into the two parental forms, the data being presented in Table XVII.

TABLE XVII.

F_2 plants from the cross of terra-cotta II and plum.

Cross	Plum	Terra-cotta II	Total
419.243 × 419.12	20	8	28
424.9 41/C.1	41	11	52
Total	61	19	80
Expected	60.0	20.0	80

In these crosses, the difference between plum and terra-cotta lies in one gene, therefore a monohybrid segregation is expected.

TERRA-COTTA II VERSUS RED. From the experimental data above shown, the genotype of terra-cotta I is regarded as $P_r m_y d_y$. In addition to this, another terra-cotta form occurs as a triple recessive, that is $p_r m_y d_y$. The evidence supporting this fact will be found in this and the following sections. Crossing red with terra-cotta II, F_1 bearing red flowers were obtained; and in F_2, simple segregation took place, as shown in Table XVIII.

TABLE XVIII.

F_2 plants from the cross of terra-cotta II and red.

Cross	Red	Terra-cotta II	Total
421.17 × 423.9	35	9	44
457.12 × 423.9	72	26	98
468.2 × 603.11	20	7	27
Total	127	42	169
Expected	126.75	42.25	169

In these crosses, the terra-cotta parent must be a triple recessive.

TERRA-COTTA II VERSUS SLATE. All F_1 plants from two crosses between terra-cotta II and slate bore slate flowers, and they segregated into two parental forms and plum flowers. The numerical data of these three forms in segregation are shown in Table XIX.

TABLE XIX.

F_2 plants from the cross of terra-cotta II and slate.

Cross	Slate	Plum	Terra-cotta	Total
423.1 × 455.4	50	11	19	80
141 × 423.9	30	7	19	56
Total	80	18	38	136
Expected	76.5	25.5	34.0	136

$\chi^2 = 2.83$ $P = 0.25$

The slate, plum and terra-cotta segregates occurred in practical accordance with a ratio of 9 : 3 : 4. Therefore, terra-cotta flowers thus produced contain both $P_r m_g d_y$- and $p_r m_g d_y$-carrying forms. The F_3 from cross 423.1 × 455.4 was examined, giving the results shown in Table XX.

TABLE XX.

F_3 of cross 423.1 × 455.4.

F_2 flower	No. Families	Slate	Plum	Terra-cotta	Total
Slate	5	65	—	—	65
	6	76	29	—	105
	5	54	—	17	71
	19	247	86	108	441
Plum	2	—	12	—	12
	4	—	29	9	38
Terra-cotta	16	—	—	368	368

By raising F_3 plants, the writer called attention to the fact that there is some difference in hue between terra-cotta segregated from plum and that segregated from slate, as shown in Plate XVI. So that, terra-cotta may be classified, possibly, into terra-cotta I bearing the gene-complex $P_r m_g d_y$, and terra-cotto II bearing the gene-complex $p_r m_g d_y$.

PLUM VERSUS RED. A red-flowered strain (No. 457.12) was crossed with a plum-flowered strain (241.35), and purple F_1 plants were obtained, the character of the hybrids being different from both parents. In F_2 only 21 plants were raised. The segregation, however, occurred in approximate accordance with the ratio 9 : 3 : 3 : 1 of dihybrid, which involves m_g and d_y. Actually F_2 consisted of 13 purple, 4 red, 3 plum and 1 terra-cotta II.

TERRA-COTTA II VERSUS PURPLE. From two crosses between terra-cotta II and purple, the writer got F_1 bearing purple flowers. In F_2, purple, red, plum and terra-cotta II were segregated as shown in Table XXI.

TABLE XXI.

F_2 plants from the cross of terra-cotta II and purple.

Cross	Purple	Red	Plum	Terra-cotta II	Total
499.25 × bpr	98	34	30	8	170
423.9 × 499.25	22	5	2	3	32
Total	120	39	32	11	202
Expected	113.6	37.9	37.9	12.6	202

$\chi^2 = 1.51$ $\quad\quad$ $P = 0.68$

The results are according to our expectation.

TERRA-COTTA II VERSUS MAGENTA. As terra-cotta II carried $p_r m_g d_y$, the F_1 hybrids obtained by crossing it with magenta bore magenta flowers. In the subsequent generation, segregation took place as to the three phenotypes, magenta, red and terra-cotta, the data of which are given in Table XXII.

TABLE XXII.

F_2 plants from the cross of terra-cotta and magenta.

Cross	Magenta	Red	Terra-cotta	Total
437 × 407.36	21	8	18	47
499.26 × D.R5	30	10	20	60
Total	51	18	38	107
Expected	*60.2*	*20.0*	*26.8*	*107*

$\chi^2 = 6.29$ $\quad\quad$ $P = 0.04$

The F_1 plants being $P_rp_rM_gm_gD_yd_y$, we may expect an F_2 segregating magenta, red and terra-cotta in the ratio 9 : 3 : 4. This expectation is approximately satisfied by the F_2 data.

BROKEN COLOUR II.

Broken colour II, including gray, drab, vinaceous I and vinaceous II, is due to another broken colour gene, duskish. Sometimes, difficulty in the distinction between vinaceous I and vinaceous II occurs in this group, as observed in the corresponding case of broken colour I. In such a case, vinaceous I and vinaceous II are gathered under a vinaceous group.

GRAY VERSUS BLUE. Two crosses between a gray strain bearing yellow leaf and a blue strain bearing green leaf, gave hybrids with green leaves and blue flowers. These hybrids resulted in normal dihybrid segregation as regards the above characters. The numerical data are shown in Table XXIII.

TABLE XXIII.

F_2 plants from the cross of gray flower with yellow leaf and blue flower with green leaf.

Cross	Green leaf		Yellow leaf		Total
	Blue	Gray	Blue	Gray	
123 × 118	24	8	8	3	43
489.10 × 487.18	30	8	10	4	52
Total	54	16	18	7	95
Expected	53.44	17.81	17.81	5.94	95

$\chi^2 = 0.38$ P ≒ 1

The segregating number in regard to the flower colour is 72 blue and 23 gray, corresponding to the ratio 3 : 1. It is sure that the dull colour is not due to a gene d_y which is linked closely with yellow leaf gene, but is due to another one d_k (previously k^2). Hence, gray refers to the gene-complex $P_rM_gd_k$.

VINACEOUS II VERSUS PURPLE. Hybrids bearing purple flowers were

given as F_1 by crossing vinaceous II with purple in both reciprocal ways. The F_2 segregation from these hybrids is shown in Table XXIV.

TABLE XXIV.

F_2 plants from the cross of vinaceous II and purple.

Cross	Purple	Red	Drab	Vinaceous II	Total
667.7×499.25	108	34	31	13	186
499.25×611.11	47	18	20	11	96
Total	155	52	51	24	282
Expected	158.6	52.9	52.9	17.6	282

$\chi^2=2.50$ $P=0.48$

These figures show the segregation of a dihybrid ratio. Vinaceous II is a duskish flower carrying two recessive genes, m_g and d_k. Drab is another duskish F_2 class, which corresponds to purple duskish or $p_rM_gd_k$.

VINACEOUS II VERSUS MAGENTA. Crossing a vinaceous strain (No. 681.15) with a magenta strain (No. 499.26), the writer got F_1 bearing magenta flowers; and in F_2, magenta, red and vinaceous were segregated in a ratio 9 : 3 : 4, as shown in Table XXV, with the numerical data of 14 F_3 families segregated in the same manner.

TABLE XXV.

Segregating data for p_r and d_k.

Source	Magenta	Red	Vinaceous	Total
499.26×681.15	45	16	23	84
Its 14 F_3 families	333	90	130	553
Total	378	106	153	637
Expected	358.31	119.44	159.25	637

$\chi^2=2.84$ $P=0.25$

In F_3 raising, vinaceous was segregated in one-fourth of the total from magenta and red F_2, respectively. Vinaceous flowers from both magenta and red F_2 so closely resemble each other that we cannot distinguish them in this cross. Vinaceous II has a gene-complex $p_r m_g d_k$, corresponding to red duskish, while vinaceous I has a gene-complex $P_r m_g d_k$, corresponding to magenta duskish.

VINACEOUS II VERSUS BLUE. The F_1 plants obtained by crossing a vinaceous II strain (No. 666.7) with a blue strain (No. 667.6) bore blue flowers, and they gave rise to an F_2 consisting of 27 blue, 9 purple, 8 magenta, 2 red, 7 gray, 3 drab and 3 vinaceous. These data are in approximate accordance with the expectation from the ratio 27 : 9 : 9 : 3 : 9 : 3 : 4, in which the three genes, p_r, m_g and d_k, are working. Though the data are small in number, it is clear that there occur four kinds of dull colours, corresponding to four pure colours, and that vinaceous II is a triple recessive as to the genes, $p_r m_g d_k$.

BROKEN COLOUR I VERSUS BROKEN COLOUR II. As described before, dusky (d_y) and duskish (d_k) are not allelomorphic. Therefore, hybrids from the crossing between both broken colours should bear normally coloured flowers. These facts were reported by the writer (1928b) and IMAI (1930). Experimental data confirming these points are collected in Table XXVI.

TABLE XXVI.

F_2 plants from the cross of broken colour I and II.

Cross	Pure colour	Broken colour	Total
424 × 241.21	37	18	55
455.21 × 419.5	19	13	32
N. k × K. 13	37	24	61
666.9 × k. 12	110	92	202
Total	203	147	350
Expected	196.9	153.1	350

A cross made between a purple strain (No. 455.2) and a drab strain (No. 419.189) gave purple flowered hybrids. In F_2, 49 purple, 14 plum and 18 drab were recorded, showing the segregation of pure and broken

colours in a ratio 9 : 7. These F_2 data, together with the F_3, confirm the genotype of the drab strain to be $p_r M_g d_y d_k$. The F_3 data are summarized in Table XXVII.

TABLE XXVII.

F_3 of cross 455.2×419.189.

F_2 flower	No. families	Purple	Plum	Drab	Total
Purple	6	362	—	—	362
	5	200	61	—	261
	5	228	—	107	335
	11	208	67	97	372
Plum	5	—	182	—	182
	5	—	196	63	259
Drab	7	—	—	446	446

The data shown above may support a supposition that the genotypes, $P_r M_g d_y d_k$, $P_r m_g d_y d_k$ and $p_r m_g d_y d_k$, may result in gray, vinaceous I, vinaceous II, respectively ; though experimental results confirming them are not yet obtained.

Genes for Flower Colours.

Summing up the experimental results above mentioned, four genes are responsible for the production of the flower colour variations, namely :

d_y (formerly k^1, dy^1)...concerns the production of dull colour ; namely, dusky.

d_k (,, k^2, dy^2)...concerns the production of another dull colour ; namely, duskish.

p_r (,, b)...results in purple, modifying the normal blue.

m_g (,, p)...modifies blue into magenta.

These four genes are active when the other three genes, C_a (formerly C^a), C and R, which are complementary for the development of the flower colour, are also present. The genetic construction of the flower colours are shown below.

Group	Hue	Genetic construction
Pure colours	Blue	$P_rM_gD_yD_k$
	Purple	$p_rM_gD_yD_k$
	Magenta	$P_rm_gD_yD_k$
	Red	$p_rm_gD_yD_k$
Broken colours I	Slate	$P_rM_gd_yD_k$
	Plum	$p_rM_gd_yD_k$
	Terra-cotta I	$P_rm_gd_yD_k$
	Terra-cotta II	$p_rm_gd_yD_k$
Broken colours II	Gray	$P_rM_gD_yd_k$, $P_rM_gd_yd_k$
	Drab	$p_rM_gD_yd_k$, $p_rM_gd_yd_k$
	Vinaceous I	$P_rm_gD_yd_k$, $P_rm_gd_yd_k$
	Vinaceous II	$p_rm_gD_yd_k$, $p_rm_gd_yd_k$

Looking over the colour series, we see that purple, magenta and slate are simple recessive to blue; red and plum are double recessive to the same; gray is either simple or double recessive; terra-cotta and drab are either double or triple recessive; and vinaceous is either double or triple or quadruple recessive, according to its genotype.

The cost of carrying out this study was defrayed by grants from Mr. TETUGORÔ HARA, Mr. HIKOICHI MOTOYAMA and the Imperial Academy. The writer wishes to express his hearty thanks for the help thus given, and also the same acknowledgment to Marquis SAIGÔ who allowed him to use an experimental farm. Furthermore, the writer takes this opportunity of thanking DR. K. MIYAKE and DR. Y. IMAI for their kind advices on many matters at the publication of this paper.

Summary.

1) So far studied, the flower colour series in Japanese morning glory (*Pharbitis Nil*) include blue, purple, magenta, red, slate, gray, plum, drab, terra-cotta I, terra-cotta II, vinaceous I, vinaceous II, white and yellow; and they may be classified under the three groups, pure colour, broken colour and white.

2) The pure colour, which is bright in hue, contains blue, purple, magenta and red. The broken colour, which is dull in hue, contains eight colours under two classes, each of which includes four dull hues, corresponding

to four normal pure colours. That is, the one, broken colour I (dusky) contains slate, plum, terra-cotta I and terra-cotta II, and the other, broken colour II (duskish) contains gray, drab, vinaceous I and vinaceous II. In both broken colour groups, however, two dull hues which correspond to magenta and red, are so slightly different that their classification is difficult in some cases, especially in the broken colour II.

3) These flower colours are due to anthocyanin pigments in the cells of the corollas, while the white group has no anthocyanin pigments in the corollas except flower tubes, in which sometimes a full amount of anthocyanin pigments occurs.

4) The results from 64 crossing experiments determined the four genes—purple (p_r) (formerly b), magenta (m_g) (formerly p), dusky (d_y) (formerly k^1, d_y^1) and duskish (d_k) (formerly k^2; d_y^2) which are active only with the co-existence of the other three complementary genes, C_a (formerly C^a), C and R, for the development of the flower colour.

5) The genetic construction for twelve colours is shown as follows: In pure colour group, blue ($P_rM_gD_yD_k$) purple ($p_rM_gD_yD_k$), magenta ($P_rm_gD_yD_k$) and red ($p_rm_gD_yD_k$); in broken colour I, slate ($P_rM_gd_yD_k$), plum ($p_rM_gd_yD_k$) terra-cotta I ($P_rm_gd_yD_k$) and terra-cotta II ($p_rm_gd_yD_k$); and in broken colour II, gray ($P_rM_gD_yd_k$, $P_rM_gd_yd_k$), drab ($p_rM_gD_yd_k$, $p_rM_gd_yd_k$), vinaceous I ($P_rm_gd_yd_k$, $P_rm_gD_yd_k$) and vinaceous II ($p_rm_gD_yd_k$, $p_rm_gd_yd_k$).

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EXPLANATION OF PLATE XVI.

All the figures are drawn in about ¾ natural size.

Fig. 1. Blue flower.
Fig. 2. Purple flower.
Fig. 3. Magenta flower.
Fig. 4. Red flower.
Fig. 5. Slate flower.
Fig. 6. Plum flower.
Fig. 7. Terra-cotta I flower.
Fig. 8. Terra-cotta II flower.
Fig. 9. White flower with coloured tube and green stem.
Fig. 10. White flower with white tube and green stem.
Fig. 11. White flower with white tube and coloured stem.
Fig. 12. Creamish flower with white tube and green stem.

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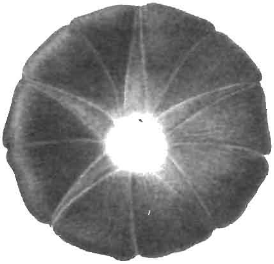

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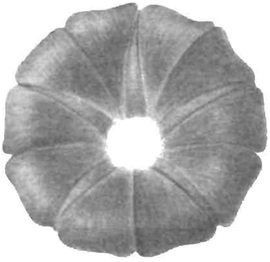

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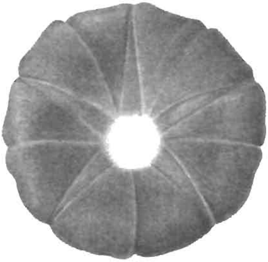
Plate XVI.



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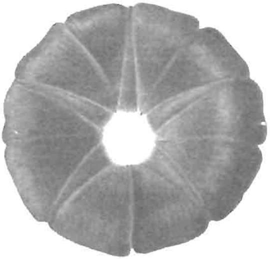

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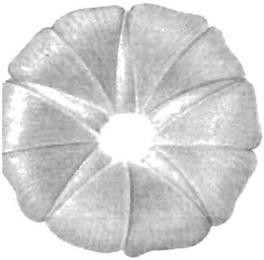

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8

Further Investigation on the Immature Stages of Some Japanese Cerambycid-beetles, with Notes on their Habits.

By

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With Plate XVII and fourteen Figures in the Text.

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Introduction.

In continuation of his previous work[1] investigation was undertaken with the immature stages of fifteen species of Cerambycid-beetles, of which three are of the Prioninae, two of the Cerambycinae and ten of the Laminae. The following is a record of those forms with notes on their life history and habits.

[1] Immature stages of some Japanese Cerambycid-beetles, with notes on their habits. This Journal, Vol. X, No. 2, 1928.

Here the author wishes to fulfil the pleasant duty of returning his heartfelt thanks to Mr. M. YANO for help and advice rendered him throughout the course of this work, and also to gentlemen for the privilege of examining the valuable material. He further desires to express his best thanks to Professor T. KABURAKI for much valuable help.

Subfamily **Prioninae.**

Tribe CALLIPOGONINI.

Megopis sinica WHITE.

(Pl. XVII, Fig. 1 and Text-fig. 1.)

Ægosoma sinicum WHITE.
Ægosoma amplicolle MOTSCHULSKY.

This species is of wide distribution, being known to occur in Hokkaido, Honshu, Bonin Island, Kyushu, Korea, Formosa and China. Up to the present nothing has been known of its immature stages. The material examined was collected by the author in Chiba prefecture and Tokyo and also by Dr. J. MURAYAMA in Korea.

IMMATURE LARVA.

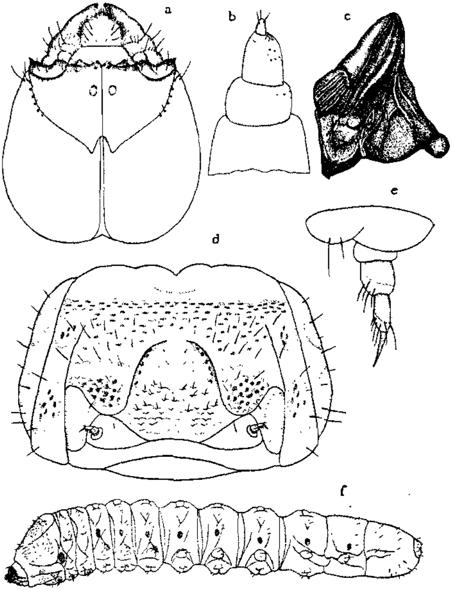
The immature larva, 16 mm. in length, is closely similar in external features to the mature larva, some differences being found in the head as follows:—Epistoma projecting over clypeus and forming a transverse belt; anterior margin of frons nearly straight and without dentation; postcondylar carina nearly straight; the disc of labrum with two pairs of setae.

MATURE LARVA.

Body white or creamy white, testaceous in the anterior portion of prothorax, fleshy, sparsely beset with brownish setae, elongate, broadest at the pro- and meso-thorax, gradually tapering to about the third abdominal segment, measuring in the largest specimen 78.3 mm. in length and 15 mm. in width.

Head capsule emerged from prothorax, light testaceous in the anterior one-third, black at the anterior margin and postcondylar carina, subquadrate, a little wider than long, with the length-breadth ratio of 1 : 1.09, with the sides convex and gradually diverging backwards, slightly emarginated at the posterior margin. Frons impressed with a well-defined median line and gently excurved frontal sutures, smooth exclusive of a small depressed area lying anteriorly on each side of the median line, be-

set on the anterior margin with five pairs of long setae, of which one occurs close to the median line and three are in front of the postcondylar carina; epistoma concave, projecting over clypeus at its lateral corners



Text-fig. 1. *Megopis sinica* WHITE.
a Head of mature larva, dorsal aspect.
b Antenna of the same.
c Left mandible of the same.
d Prothorax of the same, ventral aspect.
e Leg of the same.
f Mature larva, general aspect.

which are tooth-shaped, bearing on each side with three setae, of which *one is close to the median line and the rest are near the lateral limit;* anterior margin of frons projecting over epistoma and forming an over-

hanging carina which arises at a point just posterior to each dorsal mandibular condyle and exhibits a prominent dentation at the lateral limit and close to the median line; postcondylar carina broadly curved, longitudinally striated, acute and chitinized at the edge. Gena beset with about three long and several short setae; subfossal process projecting in a blunt tooth; epicranium provided along the frontal suture with a row of short setae; occipital foramen subquadrate, nearly straight at the anterior and lateral margins, slightly obtuse at the posterior angle; anterior foramen almost equilaterally triangular and slightly longer than the intervening tentorium. Ocelli three on each side, distinct, arranged in a row along a short carina arising from gena. Antennal ring entirely closed behind, armed with a large overhanging shield; basal skin small, not projecting from the ring; first joint cylindrical, much wider than long; second joint about 1.5 times as long as wide, slightly narrowed distads; terminal joint small, elongate, tapering distads, bearing three setigerous sensillae. Labrum testaceous, cordate, almost equal in length and width, densely setose on the margin, sparsely beset with several long setae on the disc. Clypeus transverse, trapezoidal, glabrous, with a chitinous patch in the postero-lateral region. Mandible shiny black except dark brown basal portion, obliquely emarginated and keen at the cutting edge, acute at the apex, bluntly dentate at the dorsal angle; molar face with three well-defined long oblique carinae, remarkably flattened and provided with four weak carinae near the dorsal angle; outer face with highly irregular folds as well as several short setae in the basal half. Ventral mouth-parts fleshy; lacinia short, attached to stipes; maxillary palpifer small, not distinctly separated from stipes; cardo well-defined; maxillary sclerite fleshy and considerably swollen; first joint of maxillary palpus wider than long; second joint about as long as wide; terminal joint slightly shorter than the second joint, longer than wide, narrowed distads, presenting a granulated sensory area; ligula and labial palpifer small; mentum and suhmentum fused together, chitinized, beset in the former with numerous setae, of which the posterior two are longer; terminal joint of labial palpus almost equal to or slightly smaller than that of the maxillary palpus.

Pronotum wider than long, conspicuously flattened, with distinct parallel lateral sutures in the posterior half; anterior area testaceous, fringed anteriorly with a transverse series of long setae, irregularly wrinkled, impressed with a distinct median line; middle area white, corneous, transversely wrinkled, sparsely setose, impressed with a median line anteriorly, exhibiting near each lateral limit a shallow oblique depression and a

cluster of heavy punctures; posterior area fleshy, longitudinally striated; postnotal fold distinct. Presternum slightly chitinized anteriorly, marked with setae and large punctures in scattered distribution; eusternum corneous, punctated, setose, with rounded anterior boundary which is indistinct apically.

Leg small, five-jointed; coxa fleshy, glabrous, slightly protuberant; trochanter small, scarcely visible from the exterior, with a few setae on the inner face; femur wider than long, slightly tapering distads, chitinized on the outer face, provided with a few setae on the inner face; tibia cylindrical, about 1.5 times as long as wide, chitinized and setose; tarsus chitinized, slender and spine-shaped.

Spiracle large, elongate-oval, dark castaneons at the margin; mesothoracic spiracle about 1.2 times as large as the first abdominal one; metathoracic spiracle rudimentary, represented by a small chitinous spot; first abdominal spiracle a great deal larger than the rest which are of almost equal size.

Ambulatory ampulla found in the anterior seven abdominal segments, smooth on the surface, impressed dorsally with two more or less parallel transverse and a mid-longitudinal grooves, ventrally with a single transverse groove. Epipleurum protuberant in the three segments just anterior to the anal segment; pleural tubercle well-defiued in the seventh and eighth, but not so in all the forgoing abdominal segments, with two long setae but without pit. Pleural disc visible in the first three abdominal segments, radially striated from the central pit, rather irregular in shape. Eighth abdominal segment as long as the seventh and shorter than the ninth, transversely striated; ninth abdominal segment large and projecting, almost equal in length and width, bearing twelve long setae in two transverse rows in the tergite, of which the anterior row is represented by four setae and the posterior row by eight, two setae in each epipleurum, and numerous setae sparsely arranged on the ventral surface; anal segment small, encircled with a faintly impressed line at the anterior border, trilobed by equal clefts, bearing about twelve long setae along the boundary and four on each lobe.

NOTES ON LIFE HISTORY AND HABITS.

The food plants hitherto enumerated by Y. NIJIMA[1] and S. MATSUMURA[2] are *Populus* spp., *Fraxinus mandshurica* var. *japonica*, *Abies firma*

1) NIJIMA, Y., 1913: Forest entomology. (Japanese.) p. 75.

2) MATSUMURA, S., 1921: Manual of injurious insects of Japan. (Japanese.)

and *A. sachalinensis.* Further this beetle was found to attack *Pinus densiflora, Abies firma, Mallotus japonicus* and *Salix babylonica.* So far as the author's observations in the field and laboratory are concerned, it requires at least two years for the completion of its life cycle, though different to a certain extent according to various environmental conditions, especially to the condition of food plants. Occasionally various stages of larvae evidently derived from the same batch of eggs were found in the same plant.

The condition of trees attacked by the present pest varies according to different kinds of plants. *Abies firma* and *Pinus densiflora* were found to be infested in the dead and almost highly decayed condition; *Mallotus japonicus* was found to harbour numerous larvae in the dead stumps. *Salix babylonica* was found to contain in the living state a vast number of larvae in the basal portion, seventy four larvae having been collected from the same plant.

The adult beetle emerges mostly in July and August and deposits eggs in the food plants by means of its long cylindrical ovipositor. In the case of oviposition the bark seems not to be of absolute necessity, and it was found to lack in many infested plants. The larvae directly bore inwards and upwards to the heart of tree, making a flattened tunnel in the direction of the major axis of the tree. The presence of the larvae is hardly detected from the exterior, because of the absence of excretory hole and saw dust. The frass is composed of fibrous shreds and saw dusts and is plugged in the gallery. The pupal chamber is prepared at the end of the gallery by plugging fibrous shreds to the entrance. The beetle flies out by gnawing a round hole from the pupal chamber.

The physiological injury on the living tree is not so serious, as the larvae do not feed on the cambial layer for a long time. The technical damage appears to be of some importance in some broad-leaved trees, as the wood is found to be excavated by a vast number of larvae, but it is not so in the wood of conifers such as *Abies firma* and *Pinus densiflora,* as the fresh wood never suffers the infestation.

Tribe PRIONINI.

Prionus insularis MOTSCHULSKY.

(Pl. XVII, Fig. 2 and Text-fig. 2.)

Prionus tetanicus PASCOE.
Prionus fossatus PASCOE.

This species is of wide distribution, being found in abundance in

Hokkaido, Honshu, Korea, Manchuria, North-east China and Siberia, but nothing has hitherto been reported about the larva. The material here dealt with was collected in Hokkaido.

Egg.

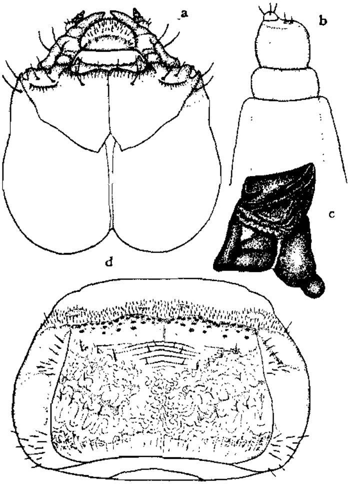
Mature ova contained in the ovarioles yellowish brown in spirit, elongate, measuring 3.7–4.1 mm. in length and 12.5–1.7 mm. in width, rounded at both ends but much larger at one end than at the other, presenting a distinct minute hexagonal reticulation.

Mature Larva.

Closely similar in general appearance to the mature larva of *Megopis sinica*, measuring in the largest specimen 82.5 mm. long by 14.9 mm. broad.

Head capsule testaceous in the anterior one-third, blackened in the anterior half of the tinted region, about 1.1 to 1.2 times as wide as long, with the sides diverging backwards and the posterior margin broadly rounded and remarkably emarginated. Frons distinctly impressed with a median line and slightly angulated frontal sutures, feebly and transversely rugose in the anterior half, beset along the anterior margin with three pairs of long and stout setae, of which two are in front of the postcondylar carinae; epistoma projecting over the clypeus, acutely dentate at the lateral limits, serrated and concave at the anterior margin, furnished with three long setae on each side, of which one is close to the median line and two near the lateral limit; anterior margin of frons carinated, longitudinally rugose, bluntly dentate at the lateral limit and slightly notched at the middle but never projecting over the epistoma. Postcondylar carina longitudinally rugose, dull at the ridge, sometimes ill-defined in the full-grown larvae. Gena with two long setae behind the pleurostoma; subfossal process prominent, forming a subacute tooth. Occipital foramen subquadrate, more or less converging posteriorly, rounded at the anterior corners, acutely emarginated posteriorly; anterior foramen triangular, a little longer than the intervening tentorium. Ocellus absent. Antennal ring with a small process anteriorly; basal skin of antenna projecting in the outer half; first joint shortly emerged, cylindrical, constricted at the base; second joint slightly longer than wide; third joint small, semispherical, bearing three sensory setae. Labrum semicircular, testaceous and densely

setose in the anterior half, castaneous and sparsely beset with long setae in the posterior half. Clypeus white, glabrous, highly transverse and trapezoidal. Mandible black, acute at the apex, angulated at the dorsal angle, oblique and curved outwards at the cutting edge; molar face strongly concave, exhibiting two long oblique carinae and a short carina near the dorsal angle; outer face rather matted, longitudinally striated, distinctly carinated along the cutting edge. Second joint of maxillary palpus wider than long; terminal joint about 1.5 times as long as wide, narrowed distads; mentum slightly chitinized, bearing numerous setae anteriorly and a few pairs of long setae posteriorly; submentum with two rather long setae.



Text-fig. 2. *Prionus insularis* MOTSCHULSKY.
a Head of mature larva, dorsal aspect.
b Antenna of the same.
c Left mandible of the same.
d Prothorax of the same, dorsal aspect.

Pronotum transverse, not highly flattened, with distinct lateral sutures in the posterior two-thirds which slightly converge forwards, distinctly impressed with a median line only in the anterior area; anterior area fringed anteriorly with a belt of pubescence, testaceous, heavily punctated; middle area corneous, sparsely setose, with transverse and vermiculate rugae, impressed near each lateral suture with two parallel and oblique furrows; posterior area fleshy, longitudinally striated, scattered with several short setae; postnotal fold well-defined. Presternum corneous, scattered with large dull-coloured punctures, sparsely setose; eusternum with the rounded anterior border, slightly corneous and sparsely setose. Leg similar in structure to that of *Megopis sinica* but relatively shorter. Spiracle small, oval, greyish brown at the

margin; one in the mesothorax about 1.5 times as large as that in the first abdominal segment which is slightly larger than the rest.

Ambulatory ampulla protuberant, faintly wrinkled on the surface, provided dorsally with two more or less convex transverse and a median impressions and ventrally with a single transverse impression which is *poorly developed in the seventh abdominal segment. Epipleurum distinctly* protuberant in the successive three segments anterior to the anal segment, provided in the anterior two with a distinct oval pleural tubercle bearing two long setae. Pleural disc visible in the first six abdominal segments but especially well-defined in the first two, represented by an orbicular area with radially striated lines from the central pore. Ninth abdominal segment rather wider than long, slightly shorter than the eighth, remarkably converging backwards, bearing in the tergite twelve long setae arranged in two transverse rows, of which the anterior row is represented by four setae and the posterior by eight, in the epipleurum two and in the sternite numerous short setae. Anal segment conspicuously projecting, more or less folded at the boundary, trilobed by equal clefts, bearing in most cases six long setae on each lobe.

NOTES ON LIFE HISTORY AND HABITS.

The food plants recorded by Y. NIJIMA[1] and S. MATSUMURA[2] are *Pinus* sp. *Cryptomeria japonica*, *Chamaecyparis obtusa*, *Fagus sieboldi* and *Ulmus* spp. The following are those determined by the author as the food plants: *Abies sachalinensis* and *Picea ajanensis* in Hokkaido and *Pinus densiflora* in Japan proper.

The adult beetle makes its appearance mostly in July and August and deposits a large number of eggs in the basal portion of dead stumps, probably independent of the presence of bark. The larva bores downwards into the root without ejecting frass or opening a hole to the exterior, and feeds especially on more or less decayed portion of the wood. Before pupation it works out a tunnel upwards to the overground portion; the emerged beetle comes out by making a round hole. The duration of life seems to be at least two years, judging from the fact that on July 20, 1929, when the adult beetles were very abundant, larvae of various sizes were found in Hokkaido feeding on the undergronnd portion of a tree. This beetle is not of economic importance, as it is a feeder of decayed wood.

1) NIJIMA, Y., 1913: Forest entomology. (Japanese.) p. 76.

2) MATSUMURA, S., 1921: Manual of injurious insects of Japan. (Japanese.)

Tribe ANACOLINI.

Psephactus remiger HAROLD.

(Pl. XVII, Figs. 3, 4 and Text-fig. 3.)

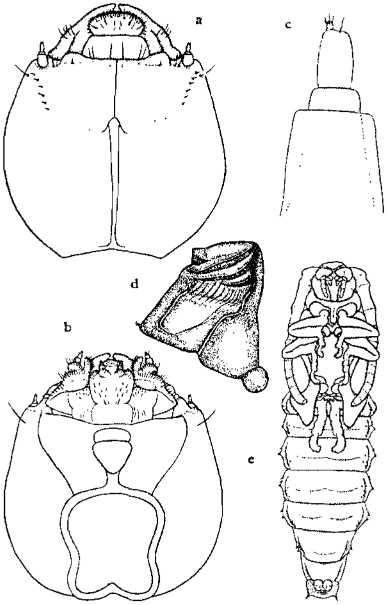
This species has been recorded from Hokkaido and Honshu, but nothing is known of its immature stages and life history. The material here dealt with was collected in the Chichibu region.

MATURE LARVA.

Body white or creamy white, testaceous in the anterior portion of prothorax, firm in the texture of integument, finely granulated, sparsely clothed with short setae, remarkably tapering from the prothorax to about the second abdominal segment, measuring in the largest specimen 52.5 mm. in length and 7.4 mm. in breadth.

Head capsule castaneous near the antero-lateral margin, a little wider than long, with the sides convex and gently diverging backwards, scarcely emarginated at the posterior margin. Frons neither carinated nor projecting at the anterior margin, with a distinct median line and ill-defined but evidently angulated frontal sutures, smooth, scattered with setigerous punctures, beset anteriorly with several long setae, of which three occur posterior to the mandibular condyle and one is close to the median line; epistoma slightly concave, neither carinated nor projecting over the clypeus, bearing three setae on each side in similar manner to that found in *Megopis sinica* and *Prionus insularis*. Gena with two long and several short setae; subfossal process large and blunt. Occipital foramen rounded anteriorly, converging posteriorly, obtusely emarginated at the posterior margin; anterior foramen triangular, with convex sides. Ocellus absent. Antenna salient; ring slightly elevated anteriorly; basal skin entirely projecting; first joint shortly emerged, slightly narrowed distads; second joint cylindrical, nearly twice as long as wide; terminal joint slightly narrowed anteriorly, about 1.5 times as long as wide, bearing three sensory setae. Labrum testaceous, suboval, about twice as wide as long, densely setose on the anterior margin, scattered with longer setae on the disc. Clypeus white, glabrous, slightly convex ou sides. Mandible blakish brown anteriorly and brown posteriorly, acute at the apex, distinctly angulated at the dorsal angle, oblique and emarginated at the cutting edge; molar face provided with three parallel oblique carinae of which the posterior one is about half as long as the rest; outer face smooth in the anterior half,

heavily rugose and beset with two setae in the posterior half. Mentum not distinctly separated from the submentum, beset with one or two pairs of setae at the posterior limit.



Text-fig. 3. *Psephactus remiger* HAROLD.
a Head of mature larva, dorsal aspect.
b Same, ventral aspect.
c Antenna of the same.
d Left mandible of the same.
e Pupa.

Pronotum wider than long, convex on the surface, with distinct lateral sutures in the posterior two-thirds; anterior area testaceous, divided anteriorly into four lobes and fringed with pubescence; middle area corneous, uniformly scattered with setigerous punctures, provided near each lateral suture with a shallow oblique groove, medially grooved posterior to the middle where the median line disappears; posterior area heavily wrinkled longitudinally, with several short setae laterally; postnotal fold distinct. Presternum partially chitinized, feebly punctated and sparsely setose; eusternum with distinct triangular border, strongly corneous, feebly rugose and sparsely setose. Leg five-jointed, relatively longer than that of *Megopis sinica* and *Prionus insularis*. Spiracle rather small, oval, greyish brown; one in the mesothorax about twice as large as that in the first abdominal segment, which is a little larger than the rest.

Ambulatory ampulla highly protuberant, especially raised laterally, finely granulated and faintly wrinkled on the surface, dorsally with two convex transverse impressions, and ventrally with a single transverse impression which is extinct at the middle. Epipleurum protuberant in the successive three segments just before the anal segment, provided in the first two with a pleural tubercle which bears two long setae. Pleural disc absent. Ninth abdominal segment longer than the eighth, longer than wide, narrowed conspicuously backwards, clothed with sparse setae which are longer and denser ventrally. *Anal segment conspicuously projecting*, folded at the border, trilobed by Y-shaped clefts, beset on each lobe with numerous setae.

PUPA.

Creamy white, finely granulated in the integument, measuring in the male 24–24.2 mm. and in the female 27.8–30 mm. in length. Head *with a small deep fovea at the vertex; antenna distinctly jointed, reaching* about the tip of the posterior tibia. Pronotum transversely rugose-striated, deeply grooved in the median line, armed with numerous erect chitinous spines bearing a seta, bluntly produced at each lateral margin; mesonotum transversely rugose-striated, deeply grooved medially, not highly produced in the scutellum; metanotum with a distinct scutellar groove, scattered with minute erect chitinous spines. Abdomen broadest at the fourth segment; tergite with numerous erect spines, impressed in the segments from the second to the fifth with two transverse grooves. Pleural zone highly protuberant, bearing several large spines, chitinized at the apex; sternite with a single transverse groove in the 2nd–7th segments; ninth segment with a pair of large and sharp caudal spines which are strongly chitinized at the apex; the area posterior to the eighth sternite *in the female represented by two large round contiguous lobes with brown* markings and in the male by a trapezoidal area which is elevated medially and highly rugose laterally; spiricle of the first abdominal segment oblique, half covered with wing pad.

NOTES ON LIFE HISTORY AND HABITS.

The adult beetle appears in late July and August. The larvae were found in large numbers excavating in the decayed *Fagus sieboldi*, packing saw dusts in the gallery and opening no excretory hole to the exterior.

From the fact that the beetles, pupae and larvae of various sizes were collected on July 22, 1928, it is safely assumed that the life cycle requires at least two years. The oviposition seems to take place independent of the bark, judging from its absence on the infested tree.

Subfamily **Cerambycinae.**

Tribe CERAMBYCINI.

Mallambyx raddei BLESSIG.

(Pl. XVII, Fig. 5 and Text-fig. 4.)

Mallambyx japonicus BATES.

This species is known to occur in Honshu, Shikoku, Kyushu and Korea and has been reported by C. SASAKI,[1] S. MATSUMURA,[2] Y. NIJIMA[3] and others as an injurious borer of the forestry and horticultural plants, but the description of the immature stages is decidedly meagre. The material here dealt with was collected by the author from Tokyo and Chiba prefectures and also by Mr. H. YUASA from Shizuoka prefecture.

EGG.

Yellow in spirit, elongate-ovoid, measuring 4.2–4.4 mm. long by 1.5–2.1 mm. broad, rounded at one end, prolonged into a thick stalk at the other end, smooth exclusive of the produced end which is marked with a large strong hexagonal reticulation, protected at the produced end with a thick opaque cap which is also heavily reticulated.

IMMATURE LARVA.

Immature larva measuring under 33 mm. in length differs from the mature larva in the following details:—Integument of body granulated; head capsule relatively longer than that of the mature larva, with the sides diverging backwards, without frontal suture; antennal ring bearing a blunt denticle; antenna salient; basal skin entirely projecting; terminal joint almost three times as long as wide, bearing a long sensory seta; accessory joint elongate and slender; mandible provided with distinct smooth tubercles; pleural disc without radial striation.

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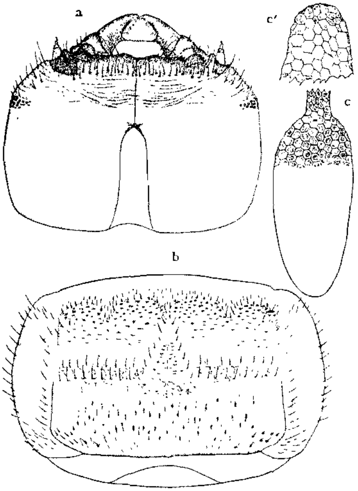
1) SASAKI, C., 1902: Injurious insects of trees. (Japanese.) Vol. II. PP. 54–56.

2) MATSUMURA, S., 1921: Manual of injurious insects of Japan. (Japanese.) Vol. II, pp. 157–158.

3) NIJIMA, Y., 1913: Forest entomology. (Japanese.) pp. 80–81.

MATURE LARVA.

Body creamy white, testaceous, strongly inclined forwards in the prothorax, fleshy, stout, very tough and smooth in the texture of integument, sparsely clothed with reddish setae of various lengths, broadest at the prothorax, tapering backwards in the thoracic segments, parallel-sided in the first eight abdominal segments, abruptly converging into a round posterior margin in the ninth segment, small and rounded in the anal segment. The largest specimen measures 62.8 mm. long by 11.2 mm. wide but just before pupation the body becomes stouter and shortened.



Text-fig. 4. *Mallambyx raddei* BLESSIG.
a Head of mature larva, dorsal aspect.
b Prothorax of the same, dorsal aspect.
c Egg.
c′ Cap of egg.

Head capsule embedded in the prothorax in the posterior half, testaceous in the emerged portion, blackened at the oral margin, nearly straight on sides, rounded at the anterior and posterior corners, nearly straight and with a shallow median emargination at the posterior margin. Frons impressed with a distinct median line and almost straight faint frontal sutures, transversely rugose-striated, provided with numerous setae anteriorly and with a pair of setae behind the middle, without postcondylar carina; epistoma weakly concave or nearly straight, bearing two long setae on each side. Gena with numerous heavy transverse rugae and dull tubercles, setose. Hypostoma slightly elevated at the anterior margin, transversely rugose-striated, scattered with setigerous punctures. Gula distinct, posteriorly divergent, especially raised near the

sides. Occipital foramen divided into two parts by a thread-like tentorial bridge; posterior or true occipital foramen subrectangular but widened posteriorly, obtusely angulated at the posterior margin; anterior foramen transversely suboval, about a quarter as wide as the posterior. Ocelli three on each side, small but distinct, arranged close to one another in a row just inside of a short carina. Antenna salient, with closed ring; basal skin large, widened posteriorly, entirely projecting from the ring; first joint about half as long as wide; second joint longer than wide; terminal joint cylindrical, about twice as long as wide, bearing a few setigerous sensillae, of which one is decidedly long; accessory joint small, elongate-conical. Labrum round-cordate, setose at the margin and dorso-lateral portions. Clypeus small, trapezoidal, glabrous. Mandible black, stout, gouge-shaped, longer than the condylar width; outer face with a transverse sulcus at the middle, smooth and with a longitudinal sulcus in the anterior half, irregularly wrinkled and provided with a few setae in the basal half. Ventral mouth parts fleshy, partially chitinized; lacinia small and rounded; maxillary articulating skin fleshy, bearing several setae; mentum about half as long as wide, provided with two or three setae on each side; submentum distinctly separated from the mentum, setose; terminal joint of labial palpus about half the basal joint, much longer than that of maxillary palpus.

Pronotum subrectangular, impressed almost entirely with lateral sutures which diverge posteriorly; anterior area testaceous, finely punctated, divided into four lobes and fringed with numerous short setae anteriorly; middle area light yellow, punctated, setose medially and posteriorly; posterior area rather fleshy, elevated, sparsely punctated and setose, transversely wrinkled; median line faintly impressed in the anterior and middle areas; postnotal fold deep. Presternum triangular, corneous, punctated, sparsely setose; eusternum with the subtriangular border which is extinct apically, corneous, punctated, setose, provided posteriorly with fleshy tubercles. Leg short, five-jointed, with sharply pointed slender tarsus which is chitinized at the apex. Spiracle oval, castaneous at the margin; mesothoracic one about 1.3 times as large as the first abdominal one; the rest smaller posteriorly.

Ambulatory ampulla of abdomen protuberant, dorsally with a median and two transverse impressions, ventrally with a transverse impression, bordered with large, fleshy, smooth and indistinct tubercles. Epipleurum protuberant in the successive three segments anterior to the anal segment, bearing numerous setae; pleural tubercle with several setae, protuberant in the first two epipleura. Pleural disc visible in the first three abdominal seg-

ments, represented by a suborbicular depressed area with striations radiated from the central pore. Anal segment trilobed by Y-shaped clefts, clothed all over with sparse setae but densely pubescent on each lobe.

PUPA.

Yellowish white, measuring 31–44 mm. long; antenna prominently swollen distally in the 3rd–7th joints; labrum triangular, depressed medially, slightly emarginated at the apex; pronotum rounded at each lateral limit, scattered with minute chitinous spines bearing a seta, raised at the posterior margin; mesonotum with the backwardly projecting scutellum; each of the first six tergites asperated in a transverse belt and in two patches in front of the belt; seventh tergites sparsely asperated; ninth tergite with two fleshy rugous projections directed backwards; the area posterior to the eighth sternite represented in the female by two large fleshy globular tubercles; spiracle greyish brown, highly elongate; first abdominal spiracle covered with wing pad.

NOTES ON LIFE HISTORY AND HABITS.

This beetle has hitherto been reported by C. SASAKI, S. MATSUMURA, Y. NIJIMA and others as a noxious borer of *Castanea sativa, Quercus acutessima* and *Quercus serrata.* Its larvae were collected by Mr. KINOSHITA from *Castanea sativa* and by the author from *Passania cuspidata* and *Quercus acutessima.* The adult beetle appears in July and August and deposits eggs in abundance mostly in the basal portion of the trunk, exhibiting no remarkable scar on the bark. The larva after feeding for a while on the cambial region burrows into the heart of tree and excavates a spaceous longitudinal gallery of an oval section. The frass is composed of coarse saw dusts and is entirely ejected from the crevice of the bark. On maturation the larva cuts out a spaceous pupal chamber mostly in the longitudinal direction and plugs the entrance with calcareous opercula, and also it cements the hole with this substance when the pupal chamber happens to stand in communication with the other.

The life cycle appears to require two years or more as many full-grown larvae were collected on September 23, 1928, from the pupal chambers from which imagos were reared in August in the next year.

The damage done is tremendous both physiologically and mechanically as the larvae attacking the living tree eat out large chambers under the bark and tunnels in the wood so as to make the trunk almost entirely hollow.

Tribe CALLIDIOPINI.

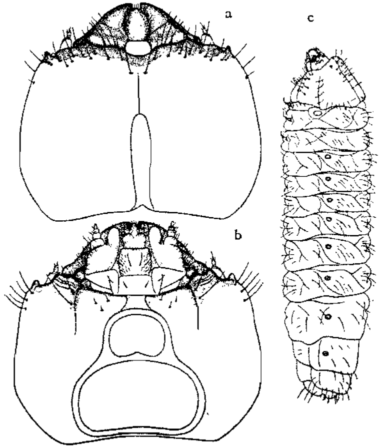
Ceresium sinicum WHITE.

(Text-fig. 5.)

This species is recorded from Kyushu and North China but nothing is known of the immature stages. Through the courtesy of Mr. H. TSAI (in China) and Dr. I. KUWANA the author had occasion to examine the material here dealt with.

MATURE LARVA.

Body yellowish white, sparsely clothed with reddish setae, rather firm in the texture of integument, stout, not highly elongate, measuring in the largest specimen 12.2 mm. long by 3.9 mm. wide, slightly tapering backwards in the first six segments, conspicuously narrowed backwards from the eighth abdominal segment.



Text-fig. 5. *Ceresium sinicum* WHITE.
a Head of mature larva, dorsal aspect.
b Same, ventral aspect.
c General view of mature larva.

Head capsule strongly castaneous near the anterior margin, about 1.3 times as wide as long, subrectangular but slightly divergent backwards, broadly rounded at the posterior corners and nearly straight at the posterior margin except for a short median emargination. Frons divided in the posterior two-thirds by a distinct median line, without frontal suture and postcondylar carina, beset anteriorly with several long setae; epistoma abruptly curved backwards in the region of clypeus, bearing three long setae on each side. Gena beset with some five long setae;

hypostoma slightly concave at the anterior margin, provided with a few short setae; gula chitinized, protuberant with concave sides; occipital foramen transversely suboval, about half the width of the head capsule; anterior foramen suborbicular, about half the width of the occipital foramen; tentorial bridge thin. Ocelli three on each side, close to one another, protected by a prominent carina. Antenna short; basal skin large, chitinized laterally, entirely projecting from the closed ring; first joint almost entirely retracted into the basal skin; second joint cylindrical, longer than wide; terminal joint slender, bearing a long sensory seta at the apex; accessory joint absent. Labrum oblong orbicular, brown at the base, setose near the margin. Clypeus small and trapezoidal. Mandible shiny black anteriorly and castaneous posteriorly, stout, gouge-shaped; outer face with a deep transeverse sulcus at the middle, smooth in the anterior half, irregularly wrinkled and provided with one or two setae in the posterior half. Lacinia large; maxillary stipes entirely chitinized; ligula slenderly produced; mentum subquadrate, setose exclusive of the median region; submentum trapezoidal, distinctly separated from the mentum, with a pair of setae; terminal joint of labial palpus elongate-cylindrical, as long as the basal joint and much longer than that of the maxillary palpus.

Pronotum rectangular, entirely impressed with a median line and lateral sutures; anterior area testaceous, fringed anteriorly with about ten long and several short setae; middle area corneous, with dull transversely wrinkled and heavily setigerous punctures, depressed along the median line; posterior area rather shiny, distinctly elevated, feebly dentate on the anterior margin, longitudinally striated; postnotal fold distinct. Presternum slightly chitinized, scattered with setae; eusternum without distinct anterior margin, slightly convex on the surface, anteriorly setose. Leg five-jointed; tarsus longer than femur and tibia, highly chitinized, crooked and sharply pointed. Spiracle nearly orbicular, testaceous at the margin; mesothoracic spiracle about 1.5 times as large as the first abdominal one which is about 1.5 times as large as the rest.

Ambulatory ampulla not highly protuberant, provided dorsally and ventrally with a single transverse impression meeting at the extremities with long and parallel lateral impressions, bordered on each side of the transverse impression with irregular fleshy tubercles. Epipleurum protuberant in the eighth and ninth abdominal segments, without pleural tubercle; pleural disc absent. Anal segment small, trilobed by equal clefts, beset with numerous setae.

PUPA.

Pale yellow, about 15 mm. long; frons with three setae on each side; antenna recurved, reaching the head; labrum longer than wide, depressed in the antero-median portion; pronotum with a small lateral tubercle on each side, scattered with setigerous chitinous spines; metanotum with a deep scutellar groove; first seven tergites with several chitinous spines; the area behind the eighth sternite in the female with two large fleshy globular tubercles.

NOTES ON LIFE HISTORY AND HABITS.

In China the larva found in the pupal chamber of the slender mulberry twig is sold for the food of cage birds. The pupal chamber is prepared by plugging fibrous shreads at the ends. It is said that the larva makes cleavages at both extremities of the pupal chamber and renders the twig easy to be broken. By rearing two larvae, imagos were obtained on June 6, 1928.

Subfamily **Lamiinae.**

Tribe MONOCHAMINI.

Psacothea hiralis PASCOE.

(Text-fig. 6.)

Psacothea flavoguttata FAIRMAIRE.

This species is of wide distribution, being recorded from Honshu, Kyushu, Formosa and North China. With regards its eggs, larvae and life history some observations were attempted by M. MAKI[1] in Formosa. The material in hand was collected in Tokyo.

EGG.

Pale yellow, elongate-cylindrical, rounded at extremities, broader at one end than at the other, smooth on the surface, measuring 4.1–4.6 mm. long by 0.9–1.1 mm. wide.

EARLY STAGE LARVA.

Small larva measuring about 15 mm. long differs from the mature larva in the following details:—Frons with three pairs of setae in a

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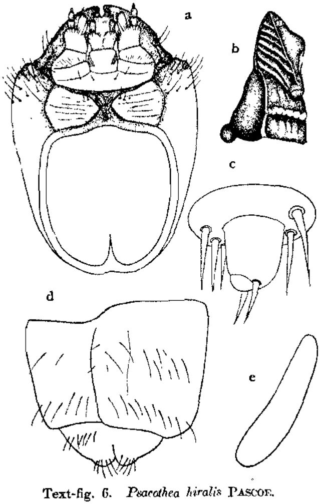
1) MAKI, M., 1916: Injurious insects of mulberry-tree in Formosa. (Japanese.) Public. Agric. Exp. Sta. Gov. Formosa. No. 90, pp. 190–218.

transverse row; hypostoma angulated at the posterior margin; gula inwardly angulated and elevated on the sides; spiracle with about six comparatively large finger-like impressions.

Mature Larva.

Body creamy white, testaceous in the prothorax, tapering from the prothorax to about the third abdominal segment, with small anal segment, measuring in the largest specimen 39 mm. long by 7.3 mm. wide.

Head capsule strongly castaneous, matted anteriorly, highly depressed, a little longer than wide, parallel or slightly convex on the sides of the anterior half and then conspicuously tapering posteriorly to end in a rounded posterior extremity. Frons with a distinct median line and ill-defined frontal sutures which are remarkably angulated at the middle, beset with three pairs of long setae in the epistoma, four pairs in a transverse row anteriorly, one pair at the middle and two at each lateral limit. Epicranial halves with a pair of long subparallel and subdorsal stripes which bear a few short setae anteriorly; gena with several long setae; hypostoma with several transverse striae, bearing two setae on each side, not distinctly elevated at the anterior margin; gula protuberant, uneven on the surface, angulated and irregular on the sides. Ocellus one on each side. Antenna short, with closed ring; basal skin projecting; terminal joint about half the length of the second, almost as long as wide, parallel-sided, bearing several sensory setae; accessory joint conical, as long as the termi-



Text-fig. 6. *Psacothea hiralis* Pascoe.
a Head of mature larva, ventral aspect.
b Mandible of the same.
c Leg of the same.
d Last two segments of the same, lateral aspect.
e Egg.

nal joint. Labrum semicircular at the anterior margin, setose in the anterior half; clypeus trapezoidal, as long as half the anterior margin, beset on each side with two or three rather long setae. Mandible black, about twice as long as the condylar width, blunt at the apex, feebly dentate at the dorsal angle, curved and emarginated at the cutting edge; molar face matted except the posterior one-fifth, provided with about six faint parallel carinae arising from a long oblique carina, transversely elevated at the boundary of the matted area; outer face rather shiny anteriorly, granulated, longitudinally rugose-striated, and beset with three setae posteriorly. Lacinia narrowed distads; maxillary sclerite with one or two setae at the boundary of the submentum; ligula mostly not bilobed at the apex; mentum about half as long as wide, bearing about three setae on each side; submentum distinctly separated from the mentum, bearing a pair of setae; basal joint of labial palpus cylindrical; terminal joint slightly tapering distads, slightly larger than that of the maxillary palpus.

Pronotum subrectangular, impressed with lateral sutures in the posterior two-thirds, with a faint median line in the anterior area; anterior area testaceous, spotted with dull-coloured punctures, fringed along the straight anterior margin with sparse numbers of setae, with a pair of setae near the middle close to the median line; middle area elevated, produced medially at the anterior margin, velured by minute chitinous granules, with large depressed punctures which are denser anteriorly, fewer and elongate posteriorly, broadly depressed and testaceous laterally, fringed anteriorly with an oblique series of setae; posterior area fleshy, velured, faintly striated longitudinally, furnished with a transverse row of short setae; eusternum of prothorax without distinct anterior margin, transversely rugose and setose in the anterior half; sternellum velured in the anterior region. Leg small, fleshy, three-jointed, furnished with several setae on the coxa and two setae at the apex of the first joint. Spiracle broadly oval, brown at the margin, with about eight small finger-like impressions on the posterior margin, about 1.5 times as large in the mesothorax as in the abdominal segments.

Ambulatory ampulla of abdomen distinctly bilobed by a median impression, provided ventrally with one and dorsally with two transverse impressions bordered with fleshy smooth tubercles. Pleural tubercles oval, bearing two long and one or two short setae but without pit. Anal segment lobed by a long straight horizontal and a very short longitudinal cleft, bearing about ten setae on the dorsal lobe, about five on each ventrolateral lobe and about fourteen in the ventral region.

PUPA.

26.5–28.3 mm. long; frons flat, bearing numerous long setae; labrum with three pairs of long setae at the base and numerous setae anteriorly; vertex nearly straight in lateral aspect, bearing a few setae; mandible with three setae; antenna swollen at the apex of each joint, curling in the female for 2.5 times and in the male for three times; pronotum scattered with setae, with a small blunt lateral projection on each side, elevated at the posterior margin; mesonotum setose, slightly swollen and angulated in the scutellum; metanotum setose, without distinct scutellar groove, slightly elevated at the anterior and posterior margins; tergites armed with long setae which are dense in the first four tergites and arranged in a transverse belt interrupted at the middle; seventh abdominal segment strongly constricted posteriorly, connected with the small eighth segment which is glabrous in the sternite; ninth tergite exhibiting a segment which is directed dorsally and chitinized at the apex, protruded ventrads and armed with several stout chitinous spines bearing a seta at the base; the area behind the eighth sternite in the female angulated at the anterior margin, medially bilobed, with two large fleshy globular tubucles, in the male represented by a trapezoidal area with a deep transverse impression. Spiracle elongate, first abdominal spiracle not covered with wing pad.

NOTES ON LIFE HISTORY AND HABITS.

Concerning the life history of this species observations were already undertaken by M. MAKI as one of the pests noxious to *Morus alba* and *Ficus carica* in formosa. So far as observations go, this species devours *Ficus carica* in Japan proper. The adult beetle gnaws, on the bark chiefly in the basal portion of the trunk, many small slit-like scars measuring about 3–4 mm. in length, and deposits one egg in each scar after working out an elongate space by means of the ovipositor. The larva feeds on the cambial region, excavating an irregular gallery under the bark and ejecting saw dusts from the crevice of the bark. On maturation larva burrows a pupal chamber, oval in sectiou, in the wood and pupates after plugging fibrous shreds at the entrance. Imago comes out by boring a round hole and eats a great deal of barks of twigs and leaves.

According to MAKI, the beetle appears in Formosa twice in a year, namely in the spring and autumn. The larval aud pupal stages last for 45 to 70 days in the first generation and far longer in the second genera-

tion owing to the hibernation in the larval stage. In Japan proper the beetle appears irregularly from May to October, but the generation seems to be once a year. The irregular occurrence of the beetles is probably due to environmental conditions during the larval stage, as the larva feeds both on living and dead trees.

Monochammus grandis WATERHOUSE.

(Pl. XVII, Figs. 6, 7 and Text-fig. 7.)

This species is distributed in the mountainous regions of Hokkaido and Honshu, bnt nothing is known of the immature stages. The material was gathered by the author from Chiba prefecture, Chichibu and Hokkaido and also given by Mr. A. KAWADA who collected it at Oyama in the prefecture of Tokyo.

EGG.

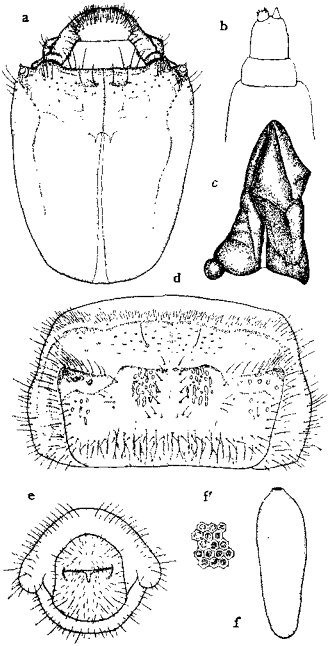
Yellowish white, measuring 5.5–6.1 mm. in length and 1.8–2.0 mm. in width, gradually tapering from one end to the other, rounded at the narrow end, exhibiting a large round hole on the exchorion at the other end, furnished all over the surface with distinct minute hexagonal riticulations, which are visible by means of magnifying glass.

MATURE LARVA.

Body white or with a slight touch of yellow, shiny, smooth in the integument, testaceous in the prothorax, clothed with numerous reddish setae, highly elongate, cylindrical but slightly depressed in the thoracic segments, conspicuously tapering from the prothorax to about the first abdominal segment, measuring in the largest specimen 89 mm. long by 11.7 mm. wide.

Head capsule exhibiting a wide transverse belt of castaneous or black *dorsally in the anterior region and ventrally in front of the occipital foramen*, moderately depressed, a little longer than wide, gradnally tapering backwards to end in a round posterior extremity. Frons occupying about anterior one-third of the whole length, distinctly impressed with a median line and slightly augulated frontal sutures, scattered all over with minute setigerous punctures, granulated and uneven in the anterior half, bearing several long setae anteriorly in a transverse row and a few long ones in

each frontal suture, beset in the epistoma with three long setae on each side. Subdorsal stripes of epicranial halves long, conspicuously excurved, furnished anteriorly with a few setigerous punctures; gena with several long setae of which two are along the hypostomal suture; hypostoma transversely striated, broadly concave and distinctly ridged at the anterior margin, bearing a few setae anteriorly; gula distinctly protuberant, remarkably narrowed backwards, smooth, with a few setigerous pits; occipital foramen nearly circular. Ocellus one on each side, large, chitinized on the surface. Antenna short with closed ring; first joint very short; second joint slightly elongate, narrowed distads; terminal joint as long as wide, rounded at the apex, bearing two setigerous and a few cylindrical sensillae; accessory joint small and conical. Labrum transversely suboval, weakly chitinized, bearing numerous setae in the anterior half. Clypeus trapezoidal, with a few short setae laterally. Mandible black, about twice as long as its condylar width, acute at the apex, dentate at the dorsal angle, broadly emarginated at the cutting edge; molar face with a well-defined carina anteriorly; outer face rather shiny, with several transverse impressed lines at the middle, abruptly swollen and beset with several setae in the basal half.



Text-fig. 7. *Monochammus grandis* WATERHOUSE.

a Head of mature larva, dorsal aspect.
b Antenna of the same.
c Right mandible of the same.
d Prothorax of the same, dorsal aspect.
e Last two segments of the same as seen from behind.
f Egg.
f′ Reticulation of egg surface.

Lacinia cylindrical; ligula bilobed apically; mentum with a few short setae on each side; submentum distinctly separated from the mentum, bearing one or two pairs of setae and several setigerous punctures; basal joint of labial palpus cylindrical, about twice as long as wide; terminal joint elongate, slightly narrowed at the apex, longer than that of the maxillary palpus.

Pronotum subrectangular, impressed with lateral sutures in the posterior half which slightly diverge anteriorly, with a median line in the anterior and posterior areas; anterior area testaceous, with shallowly impressed punctures, setose on the nearly straight anterior margin in a transverse belt, beset with a pair of setae close to the median line at about the middle; middle area elevated, velured with minute chitinous granules, impressed with large punctures which are much numerous medially, beset with several setae on each side of the median line and in front of the testaceous depressed area near lateral limits; eusternum semicircular, with faint anterior margin, setose in the anterior half; sternellum velured anteriorly. Leg rudimentary, represented by small fleshy two-jointed papillae; coxa bearing a few setae, slightly protuberant; terminal joint tubercular, bearing a few setae. Spiracle elongate-oval, greyish brown at the margin; mesothoracic spiracle about 1.5 times as large as the first abdominal one which is about 1.2 times as large as the rest.

Ambulatory ampulla of abdomen provided dorsally with two transverse and convex grooves, ventrally with a single groove, bordered with large round tubercles bearing numerous chitinous granules. Epipleurum protuberant in the first eight abdominal segments, bearing numerous setae; pleural tubercle oval, with two chitinous pits and several setae of which two are long. Anal segment semicircular in dorsal aspect, lobed by a long straight horizontal and a very short vertical cleft, beset all over with numerous short setae.

PUPA.

Male specimen 34.8 mm. long and 9.2 mm. across at the base of elytra; frons distinctly impressed with a median line, setose; labrum provided with numerous setae anteriorly and with three or four setae at the base; vertex nearly straight in lateral aspect, bearing a few setae anteriorly; mandible with four setae; antenna roundly curling for three times and half; pronotum scattered with setae, slightly ridged at the anterior and posterior margins, with a large blunt lateral projection on each side; mesonotum setose, protuberant, rounded in the scutellum; metanotum

setose, without distinct scutellar groove, slightly ridged at the anterior and posterior margins. Abdomen remarkably widened at the third segment, armed with a transverse interrupted band of dense stout setae in the first six tergites; seventh tergite setose only posteriorly; eighth tergite small, provided with chitinous spines bearing a seta at the base; ninth tergite giving rise to a large acute chitinous caudal spine; eighth sternite with two setae; the area behind the sternite in the male trapezoidal, with a large transverse fovea at the center; spiracle elongate-oval; first abdominal spiracle oblique and exposed.

NOTES ON LIFE HISTORY AND HABITS.

As to the life history of this species nothing is known, besides the fact that it is an injurious borer of *Abies firma*, *A. sachalinensis* and *Picea ajanensis* (S. MATSUMURA[1] and Y. NIJIMA[2]). The author collected some larvae from *Abies firma*, *A. sachalinensis* and *Pinus densiflora*. The imago appears extending from June to August and deposits many eggs under the bark of the newly fallen or dead trunk or stump by inserting an egg from a slit-like scar gnawn by itself. The larva, after eating out a spaceous gallery under the bark, enters into the heart of wood by boring a tunnel, oval in section, which turns to the direction of the major axis of the tree and pupates at the end of the gallery, but sometimes it prepares an oval pupal cell under the bark by plugging fibrous shreds. The frass is composed of coarse fibrous shreds and is thrown out from a crevice on the bark which is made by enlarging the egg incision. The life cycle has not yet been followed, but it seems to require at least two years, judging from the following observations:—In late July many full-grown and half-grown larvae were collected in Chiba prefecture, Chichibu and Hokkaido; three pupae were found on May 6, 1920, of which two emerged on June 12 and July 10 in the same year. The damages on the forestry trees and products are very serious as this borer rapidly attacks the newly fallen and dead trees. According to NIJIMA, not only does this pest attack vigorous *Abies sachalinensis* in Hokkaido, but also it causes a serious damage to the newly planted conifers, especially the pine and larch, by feeding on the bark to a considerable extent.

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1) MATSUMURA, S., 1920: Manual of injurious insects of Japan. (Japanese.) Vol. II, pp. 143-144.

2) NIJIMA, Y., 1913: Forest entomology. (Japanese.) pp. 86-87.

Monochammus sartor FABRICIUS.

(Pl. XVII, Fig. 8 and Text-fig. 8.)

Monochammus sartor var. *quadrimaculatus* MOTSCHULSKY.

Monochammus quadrimaculatus MOTSCHULSKY.

Monochammus bosenmülleri JACOBSON.

This species is of wide distribution, being recorded from Saghalien, Hokkaido, Korea, Siberia and Europe. Judging from the descriptions given by SCHIOEDTE[1], SAALAS[2] and ESCHERICH[3], Europian larva appears to differ from Japanese one in some respects. The material in hand was collected by the author in Hokkaido and Saghalien.

EGG.

Closely resembles that of *Monochammus grandis* but different in the following points:—Size varying from 4.6 to 5.4 mm. in length and from 1.7 to 1.8 mm. in width; surface more indistinctly reticulated with irregular lines.

FIRST STAGE LARVA.

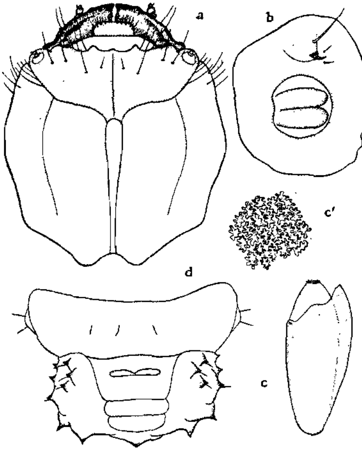
Small larva measuring 5.5–10 mm. in length differs from the larger form in the following points:—Head capsule light castaneous in the emerged portion, nearly as long as wide or slightly elongate, conspicuously widened at the middle, emarginated at the posterior margin; frons with a pair of small blunt denticles on the anterior margin, beset with three pairs of setae anteriorly; hypostoma with two pairs of small denticles on the anterior margin, distinctly bisected by a median impression; occipital foramen augulated at the anterior margin; mandible castaneous, dark anteriorly, often with a small indistinct tubercle anteriorly on the outer face, granulated and with two setae in the posterior half; mentum and submentum with a pair of setae. Pronotum shiny testaceous and transversely striated in the anterior half, brown with minute chitinous granules in the posterior half; eusternum of prothorax with distinct semicircular anterior border, beset with several long setae; mesonotum

1) SCHIOEDTE, J. G., 1875: De metamorphosi Eleutheratorum, Naturh. Tiddskrift., III, 10, pp. 434–436.

2) SAALAS, U., 1923: Die Fichten Käfer Finnlands II. Ann. Acad. Scient. Fennicae. Ser. A, Vol. 22, No. 1., pp. 411–413.

3) ESCHERICH, K., 1923: Die Forstinsekten mitteleuropas II. pp. 225–228.

with minute chitinous granules anteriorly; spiracle with two backwardly projecting lobes, provided dorsally with a stout chitinous spine bearing a seta at the base; pleural tubercle with two setae but without pit; anal segment with six long setae on the dorsal lobe and three on each ventro-lateral lobe.



Text-fig. 8. *Monochammus sartor* FABRICIUS.
a Head of first stage larva, dorsal aspect.
b Spiracle of the same.
c Egg.
c' Reticulation of egg surface.
d Last three segments of male pupa, ventral aspect.

IMMATURE LARVA.

The larva over about 10 mm. in length differs from the first stage larva in possessing round-oval spiracle and more numerous setae on the head capsule and body.

MATURE LARVA.

Very closely similar in general feature to that of *Monochammus grandis*. The largest specimen measures 72 mm. long by 10.3 mm. across at the prothorax. Head capsule closely allied to that of *Monochammus grandis* except the following details:—Length-breadth ratio on an average of twenty head capsules is 1.162 ± 0.008 mm., whereas it is in *Monochammus grandis* 1.213 ± 0.012 mm.; dorsal face highly flattened; hypostoma more convex on the surface; mandible presenting a very distinct boundary between the anterior and posterior halves on the outer face especially at the ventral limit, which is represented by numerous short vertical striae, whereas in *Monochammus grandis* such markings are almost absent, and also the swelling in the posterior half of the outer face is larger and more protuberant.

PUPA.

Two specimens measuring 35.5–37 mm. long by 9–9.7 mm. across at

the base of elytra exhibits a great resembrance to *Monochammus grandis* except the following details:—Vertex roundly curved in lateral aspect; antenna curling nearly for three times in the male and once in the female; pronotum without ridge at the anterior margin, with highly acute lateral projections; scutellum highly protuberant, rounded at the margin, bilobed with a distinct median depression; abdomen nearly parallel-sided in the first four segments; caudal spine more acute and longer; area behind the eighth sternite in the female small, trapezoidal, bilobed, with two large fleshy globules, and in the male subrectangular, with a large transverse fovea at the center; first abdominal spiracle covered with wing pad.

NOTES ON LIFE HISTORY AND HABITS.

The life history has been observed by many authorities in Europe such as ESCHERICH, KOCH[1], SAALAS, etc., as one of the injurious borers of spruce and pine trees. The food plants in Japan are *Abies sachalinensis* and *Picea ajanensis*, as already reported by S. MATSUMURA[2]. The imagos appear in July and August in abundance and deposit eggs under the bark of newly cut logs and sometimes of living trunk. On oviposition the female beetles gnaw in irregular manner many lenticular scars of some 8 mm. in length and insert one egg in each scar at a depth of some 3–4 mm. under the bark, keeping the major axis of the egg in transverse to the direction of the scar. The imagos swarm during the sunny day-time in the freshly logged area and the stock field of logs and crawl over the trunk singly or in copula.

The larva eats out for some time under the bark, excavating a broad and irregular gallery in the sapwood region and barrows a tunnel of oval section into the heart of tree and then in the direction of the major axis of the trunk. A considerable amount of fibrous shreds are thrown out from the enlarged hole of egg incision on the bark. It is noticeable that the larva makes a large creaking sound in the gallery. Pupation takes place in the pupal chamber which is built at the end of the gallery by packing a considerable amount of fibres at the entrance. The head of the pupa is directed to the end of the pupal chamber, and on emergence the beetle bores a rounnd hole directly from the pupal chamber to the exterior and crawls out.

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1) KOCH, R., 1928: Bestimmungstabellen der Insecten an Fichten und Janne nach Frassbeschädigungen. pp. 112–119; ditto, pp. 134–135.

2) MATSUMURA, S., 1929: Manual of inqurious insects of Japan. (Japanese.) Vol. 2, pp. 144–145.

The life cycle can be proved to be one year by the author's rearing experiment, but it is also obvious that it requires two years or more under unfavorable environmental conditions, from a consideration of the fact that the author collected numerous larvae in the various stages of growth during a short period from late July to early August when the adult beetles were very abundant.

The economic importance of this pest is very tremendous, as it commits physiological and serious mechanical damages by attacking both fresh logs and living trees.

Monochammus tesserula WHITE.

(Pl. XVII, Fig. 9 and Text-fig. 9.)

This species is of wide distribution, being known to occur in Honshu, Kyushu, Formosa and China. Some observations were attempted by M. YANO on its life history and habits. The description of immature stages are, however, left untouched. The material here dealt with was collected by the author in Chiba prefecture and also by Messrs. A. KAWADA and H. YUASA in Tokyo.

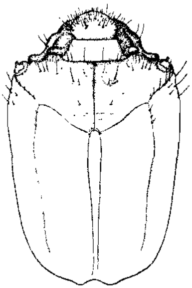
MATURE LARVA.

Closely allied in general appearance to other *Monochammus*-larva, exclusive of the small anal segment which is slightly visible in dorsal aspect. Largest specimen 43.5 mm. in length and 9 mm. in width.

Head capsule highly depressed, dark castaneous in the antero-dorsal region and in front of the occipital foramen, about 1.3 times as long as wide, with sides parallel or slightly convex in the anterior half and conspicuously convergent in the posterior half; the posterior margin rounded, shortly emarginated medially. Frons with a distinct median line and ill-defined angulated frontal sutures, bearing several short setae anteriorly and several long setae of which three pairs are in the epistoma, four pairs in a transverse row anteriorly and one pair near the middle; subdorsal stripes of epicranial halves long, excurved, beset with a few short setae anteriorly; hypostoma conspicuously curved, distinctly ridged at the anterior margin, beset with several long setae; gula protuberant, remarkably divergent forwards, smooth but with a few small pits. Ocellus one on each

[1] YANO, M., 1913: The research on the death of the pine forests in the prefecture of Nagasaki. (Japanese.). Sanrin-koho. No. 4, Suppl. pp. 1–4.

side, slightly chitinized on the surface. Terminal joint of antenna small, semispherical, bearing about four setigerous sensillae; accessory joint conical nearly as long as the terminal. Labrum transverse, suboval, setose in the anterior half and on the lateral margins; clypeus trapezoidal, with a few short setae near the lateral limit; mandible similar to that of *Monochammus grandis;* mentum provided with two pairs of long and eventually with a few short setae; submentum distinctly separated from mentum with a pair of long setae and a few short ones. Spiracle round-oval.



Text-fig. 9. *Monochammus tesserula* WHITE.
Head of mature larva, dorsal aspect.

PUPA.

Resembles other *Monochammus*-pupa, measuring 14–26.9 mm. long by 3.6–7.2 mm. across at the base of elytra; vertex rounded in lateral aspect; antenna curling in the male for three times and half and in the female for 1.5–3.5 times; pronotum ridged posteriorly, medially depressed, provided with rather acute lateral projections; scutellum rather angulated; metanotum ridged at the anterior and posterior margins, with an ill-defined scutellar groove; first seven tergites sparsely beset with an interruped belt of chitinous spines; eighth tergite with three or four spines; caudal spine stout and acute, chitinized at the apex; spiracle broadly oval, first abdominal one half covered with wing pad; the area behind the eighth sternite similar to that of *Monochammus sartor*.

NOTES ON LIFE HISTORY AND HABITS.

The imago appears in June and July and oviposits under the bark of *Abies firma, A. smithia, Picea exelsa, P. morinda, Pinus densiflora, P. banksiana, P. strobus,* and also, according to Mr. H. YUASA, *Cedrus deodara.* Eggs are laid in abundance extending from the basal portion of the trunk to the branch of some 3 cm. in diameter. According to M. YANO several eggs are laid in a shallow scar gnawn on the bark. The larva, after feeding for a long time under the bark, excavates a tunnel, oval in section, into the heart of wood, which turns to the direction of the major axis of the infested portion of the tree. The frass which is

composed of fibrous shreds is packed in the gallery under the bark. Pupation takes place at the end of the tunuel after preparing a pupal cell by packing fibrous shreds at the entrance. The damage on the forestry tree is serious as this pest commits both physiological and technical damages by attacking either living or dead trees. The infestation in the living tree has been found in the imported species and replanted trees. For the completion of life cycle this beetle requires one year as stated by YANO.

Dihammus luxuriosus BATES.

(Pl. XVII, Figs. 11-12 and Text-fig. 10.)

Monochammus luxuriosus BATES.
Haplohammus luxuriosus BATES.

This species is of wide distribution in Japan, being recorded from Hokkaido, Honshu, Kyushu and Quelpart Islands. The egg, larva and pupa were briefly placed on record by T. ISHII.[1] The specimens here dealt with are those which were collected by the author in Hokkaido and also by Messrs. S. KINOSHITA and H. YUASA.

MATURE LARVA.

Body white with a slight touch of yellow, testaceous in the prothorax, firm in the texture of integument, closely allied to *Monochammus*-larva in general appearance excepting the high projection of the anal segment, measuring in the largest specimen 79.3 mm. long by 10.6 mm. wide at the prothorax.

Head capsule testaceous in the anterior two-thirds, castaneous and granulated in the dorsal one-fifth and in the entire region anterior to the occipital foramen, depressed, almost 1.2 times as long as wide, parallel-sided in the anterior two-fifths, then strongly convergent backwards to end in a nearly straight posterior extremity. Frons shorter than one-third the length of head capsule, with a distinct median line and augulated frontal sutures, transversely rugose, scattered with numerous setigerous punctures, provided with several long setae, of which three pairs are in the epistoma, and four or five pairs are arranged in a transverse row in the anterior region. Epicranial halves with markedly excurved subdorsal stripes which present several setigerous pits anteriorly. Hypostoma broadly curved and

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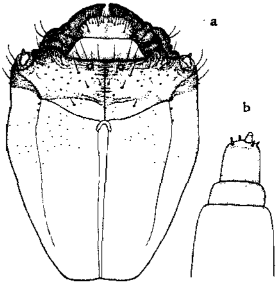
1) ISHII, T., 1920: On two injurious insects of *Aralia cordata*. (Japanese.) Byochugai-zasshi, Vol. 7, pp. 986-689.

ridged at the maxillary margin, transversely rugose-striated, scattered with setigerous punctures, beset anteriorly with a few long setae; gula rather weakly protuberant, distinctly bordered only anteriorly, with a pair of large setigerous pits and an elongate median depression. Occipital foramen rather converging backwards. Ocellus one on each side, slightly chitinized on the surface. Antennal ring closed but angulated backwards; basal skin small, exposed only on the dorsal side; first joint very short; second joint a little elongate, cylindrical, rounded at the apex, bearing a few cylindrical sensillae; third joint small, subconical; accessory joint absent. Labrum suboval, testaceous in the posterior half, medially depressed, densely setose in the anterior half. Clypeus transverse, shorter than half the anterior width, glabrous, chitinized at base. Mandible black, about 1.5 times as long as its condylar width, acute at the apex, right-angled at the dorsal angle, sharply edged and emarginated at the cutting edge; molar face provided with a well-defined oblique carina which is anteriorly swollen into a dull ridge parallel to the base in the posterior region; outer face with deep transverse impressions at the middle, rather shiny, with a longitudinal impression in the anterior half, conspicuously swollen and with granules and two setae in the posterior half. First and second joints of maxillary palpus elongate; third joint elongate-conical; ligula large, slightly emarginated at the anterior margin: mentum with five or six long setae on each side, of which three are remarkably long; submentum distinctly separated from mentum with a pair of setae and several small pits.



Text-fig. 10. Dihammus luxuriosus BATES.
a Head of mature larva, dorsal aspect.
b Antenna of the same.

Pronotum similar in structure to that of *Monochammus*-larva except the following details:—Median line distinct for whole length; middle area not distinctly elevated, dull-coloured, velured but without chitinous asperities, impressed with numerous pits of varying shapes and sizes; posterior area velured similar to the middle area, interspersed with elongate setigerous pits. Epipleurum of prothorax testaceous with large punctures; presternum densely beset with short setae; eusternum fairly distinct and

rounded at the anterior border, punctated, setose in the anterior half; sternellum velured and longitudinally striated in the anterior half. Leg reduced, composed of two joints; coxa scarcely protuberant, with about twelve setae; second joint short with two setae. Spiracle elongate-oval; mesothorasic spiracle about 1.5 times as large as the first abdominal one, which is a little larger than the rest.

Ambulatory ampulla of abdomen similarly constructed to that of *Monochammus*-larva; pleural tubercle oval, bearing two long and one or two short setae as well as two pits. Anal segment projecting, beset with numerous setae, trilobed by equal clefts, the dorsal two clefts enclosing a very obtuse angle but not in the same straight line.

NOTES ON LIFE HISTORY AND HABITS.

The food plants, so far as the author can learn, are *Abies sachalinensis* (Y. NIJIMA[1]), *Kalopanax ricinifolius* (S. MATSUMURA[2]), *Aralia cordata* (S. KINOSHITA, T. ISHII and S. TAKAHASHI[3]) and also *Fatsia japonica* (S. KINOSHITA). The author collected larvae from *Kalopanax ricinifolius* and *Tilia cordata* in Hokkaido. It is of striking interest that the mode of its attack varies in different kinds of food plants. In *Kalopanax ricinifolius* and *Tilia cordata* the larva feeds on an extensive area of sapwood just beneath the bark of dead trees and excavates an oval tunnel into the heart of the wood to make a pupal chamber. According to ISHII and TAKAHASHI, one egg is deposited in the egg incision guawn in the basal portion of *Aralia cordata*, and the larva bores a longitudinal gallery in the stem and pupates in the underground portion of the stem after packing fibrous shreds. In *Fatsia japonica* the larva is said to feed for some time under the bark of living tree and then bores a long gallery in the stem. The generation is known to be in *Aralia cordata* once a year. The larva is said to hatch out twelve days after oviposition, hibernates in the middle of the stem, pupates in June and flies out in July. In *Kalopanax recinifolius* and *Tilia cordata*, so far as observations go, the life cycle appears to require two years or more, as in late July of 1929 several large larvae were found in Hokkaido still feeding uuder the bark.

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1) NIJIMA, Y., 1913: Forest entomology. (Japanese.). p. 86.

2) MATSUMURA, S., 1920: Manual of injurious insects of Japan. (Japanese.) Vol. 2, pp. 142–143.

3) TAKAHASHI, S., 1929: Injurious insects of horticulture and their control. (Japanese.) Yengei-gaichu Kujo-yobō-ho. pp. 110–112.

Dihammus cervinus HOPE.

Dihammus fraudator BATES.

Dihammus fulvicornis PASCOE.

Dihammus sejunctus BATES.

This species is of very wide distribution, being known to occur in Hokkaido, Honshu, Kyushu, Korea, Quelpart Islands and India. The immature stages have been thoroughly described by J. C. M. GARDNER[1] and briefly by C. SASAKI.[2] The material serving as basis for the following record is represented by a single larva which was kindly placed by Mr. E. NINOMIYA in his hand for examination.

MATURE LARVA.

Closely allied to that of *Dihammus luxuriosus*, but can be distinguished by the following points:—Sides of head capsule slightly diverging for some distance and then narrowed posteriorly, ending in a round posterior margin; occipital foramen round-oval, not conspicuously narrowed backwards; the middle area of pronotum asperated with minute brown chitinous tubercles besides some interspersed glabrous spots; presternum of prothorax with a few setae; tubercles of ventral ampullae of meso- and meta-thorax not distinct, contiguous into two transverse belts; pleural tubercle with two setae and two pits. Body about 30 mm. in length.

NOTES ON LIFE HISTORY AND HABITS.

According to C. SASAKI, this borer infests *Daphniphyllum macropodum*. The adult beetle appears in June and July and oviposits in the living tree. The larva feeds for some time on the sappy portion under the bark and excavates rather long galleries in the heart of the tree in the direction of the major axis of the stem. The duration of life cycle is one year, and the larva pupates in February and March. The damage committed on the tree is so serious as to cause the death of the combial tissue.

Melanauster chinensis FÖRSTER.

(Pl. XVII, Fig. 12 and Text-fig. II.)

This species is very common in Japan, being known to extend from

1) GARDNER, J. C. M., 1927: Identification of immature stages of Indian Cerambycidae, II. Indian Forest Records. Vol. 13, pt. 2.

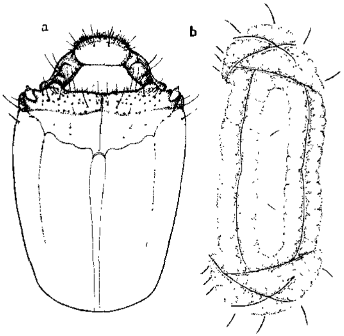
2) SASAKI, C., 1902: Injurious insects of trees. (Japanese.) Vol. 3, pp. 84-86.

Hokkaido in the north to Formosa in the south. Up to the present the larva has been meagrely described by M. MAKI.[1] The material was collected by the author in Chiba and Tokyo prefectures and also was kindly placed at my disposal by Messrs. S. TAKAHASHI and H. TSAI.

MATURE LARVA.

Body white or with a slight touch of cream, granulated in the integument, allied in general appearance to that of *Monochammus*-larva, with a projecting anal segment which is semicircular in dorsal aspect, measuring in the largest specimen 47 mm. long by 7.5 mm. across at the prothorax.

Head capsule testaceous in the anterior half, castaneous near the oral margin, moderately depressed, about 1.4 times as long as wide, with sides parallel or slightly excurved in the anterior half and then gradually converging into the broadly rounded posterior margin. Frons occupying about the anterior quarter of head capsule, with distinct median and frontal sutures, of which the latter is notched at the middle and beset with several short setae anteriorly, smooth, with numerous punctures, about four pairs of long setae in the anterior region and three pairs of long setae in the epistoma, of which one is close to the median line and two are near each lateral limit. Subdorsal stripes of epicranial halves slightly excurved, without seta. Hypostoma remarkably curved at the anterior margin, smooth, bearing a pair of long and a few short setae; gula castaneous, nearly parallel-sided, protuberant especially on the sides, beset with two short setae. Occipital foramen oval. Ocellus one on each side, slightly chitinized on the surface. Antennal ring closed but con-



Text-fig. 11. *Melanauster chinensis* FÖRSTER.
a Head of mature larva, dorsal aspect.
b Dorsal abdominal ampulla of the same.

[1] MAKI, M., 1916: Injurious insects of mulberry-tree in Formosa. (Japanese.) Public. Agric. Exp. Sta., Gov. Formosa. No. 90, pp. 190–218.

spicuously extended and angulated posteriorly; basal skin projecting; second joint nearly as long as wide; third joint small, rather wider than long, bearing several setigerous sensillae; accessory joint conical, almost as long as the third joint. Labrum semicircular at the anterior margin, constricted near the base, densely setose in the anterior half. Clypeus glabrous. Mandible black but brown near the base, about 1.5 times as long as its condylar width, dull at the apex and dorsal angle; molar face with an oblique carina anteriorly and with a deeply impressed line posteriorly, which is parallel to the base; outer face matted and with a few faint longitudinal striae in the anterior half, granulated and with two setae in the basal half. Two basal joints of maxillary palpus longer than wide and subcylindrical; third joint elongate, gradually narrowed distads; maxillary sclerite with several chitinous spots at the boundary near the submentum; ligula not bilobed at the apex; mentum with five or six setae on each side; submentum distinctly separated from the mentum, with a pair of setae and several spots; basal joint of labial palpus highly elongate; terminal joint longer than that of maxillary palpus.

Pronotum closely allied to that of *Monochammus*-larva but with the anterior margin conspicuously rounded and fringed with a belt of pubescence; anterior area protruded, rounded at the margin, with numerous dull-coloured spots, beset with a longitudinal series of setae on each side close to the median line; middle area with a dense series of setae in front of the lateral depressed area; median line distinct only in the anterior area. Presternum of prothorax pubescent; eusternum with semicircular anterior border which is extinct at the apex, beset with numerous setae anteriorly; sternellum velured with minute chitinous granules in the entire area. Legs small, three-jointed; coxa rather protuberant, bearing a few setae; second joint about as long as wide, bearing two setae; terminal joint minutely tubercular. Spiracle broadly oval, light brown at the margin, mesothoracic spiracle about 1.5 times as large as the first abdominal one, the rest smaller posteriorly.

Ambulatory ampulla of abdomen furnished dorsally with two convex impressions and ventrally with a single impression bordered with ill-defined tubercles which exhibit a contiguous and minutely asperated belt on each side of the impression. Epipleurum not highly protuberant; pleural tubercles with two setae and two pits. Anal segment trilobed around the anus, with almost equal clefts, of which two dorsal clefts involve an obtuse angle but not in the same line, beset on each lobe with twelve to fourteen setae and in the ventral area with about twelve setae.

NOTES ON LIFE HISTORY AND HABITS.

This borer has up to the present been treated by many authors, such as C. SASAKI[1], Y. NIJIMA[2], S. MATSUMURA[3], M. MAKI, S. TAKAHASHI[4], etc. as an injurious pest of *Morus alba*, *Citrus* spp., *Salix* spp., *Malus pumila* and *Melia japonica* in Japan proper as well as of *Melia azedarach*, *Cajanus indicus* and *Casuarina equisetifolium* in Formosa. The author has collected some larvae from *Platanus orientalis* and *Mallotus japonicus*. A single larva placed at my disposal by Mr. H. TSAI was collected from *Populus alba* in China.

The adult beetle appears in July and August and deposits eggs chiefly in the basal portion of the living trunk, although it also oviposits in the higher portion when the trunk is of a large diameter. When in oviposition, according to MAKI's observation, the beetle gnaws a T-shaped scar on the bark and lays one egg in each incision. The larva bores into the wood, after feeding for a short time under the bark, and excavates a longitudinal gallery, and also it opens a round excretory hole and ejects a considerable amount of saw dusts in the form of small cylindrical pellets. The life cycle is said to be completed for twelve or thirteen months in Formosa and two or three years in Japan proper.

Eupromus ruber DALMAN.

(Pl. XVII, Fig. 13 and Text-fig. 12.)

Melanauster championi WAITE.

Cyriocrates ruber LEWIS.

Cyriocrates sieboldi GUER.

Inhabiting Honshu, Shikoku, Kyushu and China is found the present species, on the immature stages of which nothing has hitherto been recorded. The following description is based on the material collected in Tokyo, and kindly placed at my disposal by Dr J. MURAYAMA. The latter material was collected in the prefecture of Ehime.

1) SASAKI, C., 1902: Injurious insects of trees. (Japanese.) Vol. 2, pp. 176–178.

2) NIJIMA, Y., 1913: Forest entomology. (Japanese.) pp. 78–79.

3) MATSUMURA. S., 1921: Manual of injurious insects of Japan. (Japanese.) Vol. 2, pp. 150–151.

4) TAKAHASHI, T., 1929: Injurious insects of crops. (Japanese.) pp. 271–272; 1929: Injurious insects of horticulture and their control. pp. 283–286.

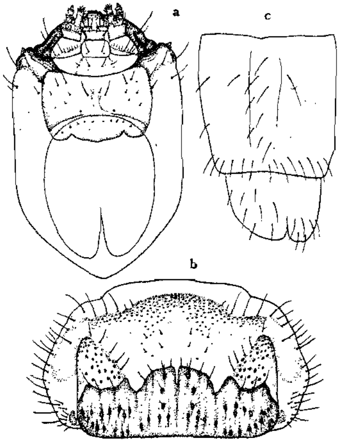
Immature Stage.

Different from mature larva in possessing the head capsule which is rounded at the posterior margin.

Mature Larva.

Resembles *Monochammus*-larva in general appearance except for its highly projecting anal segment, measuring in the largest specimen 54 mm. long by 7 mm. across at the prothorax.

Head capsule castaneous dorsally near the anterior margin and ventrally in the entire area in front of the occipital foramen, more than 1.35 times as long as wide, parallel-sided, converging posteriorly into the slightly angulated posterior margin. Frons with a distinct median line and nearly straight frontal sutures, beset all over with short setae, possessing four pairs of long setae anteriorly and three long and eventually one or two short setae on each side of the epistoma. Subdorsal stripes of epicranial halves almost parallel, bearing a few short setae anteriorly. Hypostoma concave at the anterior margin, produced backwards into the oval occipital foramen exhibiting three rounded lobes, beset with a pair of long and several short setae; gula not protuberant, represented by a narrow light zone. Ocellus one on each side, rather protuberant. Antenna short, with closed ring; basal skin exposed merely on the dorsal side; first joint very short; second joint about as long as wide; terminal joint small, shorter than wide, bearing a few setigerous sensillae; accessory joint elongate-



Text-fig. 12. *Eupromus ruber* Dalman.
a Head of mature larva, ventral aspect.
b Prothorax of the same, dorsal aspect.
c Last two segments of the same, lateral aspect.

conical, longer than the terminal. Labrum suboval, entirely setose; clypeus shorter than half its anterior margin, bearing several short setae at each lateral limit. Mandible black, nearly 1.5 times as long as its condylar width, acute at the apex, dentate at the dorsal angle, strongly curved at the cutting edge; molar face with a highly prominent carina parallel to the cutting edge anteriorly, depressed in the postero-median region; outer face rather shiny, with several faint longitudinal striae in the anterior half, granulated, longitudinally folded, beset with two long and a few short setae in the posterior half. Maxillary palpifer and stipes each with two long and several short setae; maxillary sclerite with a long and a few short setae at the boundary near the submentum; two basal joints of maxillary palpus as long as wide, thickened anteriorly; terminal joint elongate, narrowed forwards and rounded at the apex; ligula rounded at the anterior margin; labial palpifer with a long and a few short setae; mentum much shorter than half the width, provided with a pair of long setae and eventually a few short ones; submentum with a pair of setae; terminal joint of labial palpus longer than that of maxillary palpus.

Pronotum impressed with lateral sutures except for a short anterior portion; anterior area conspicuously rounded, beset with several long setae on the anterior margin, testaceous, punctated, distinctly divided by a median line; middle area not chitinized, without distinct median line, broadly depressed and punctated near the lateral sutures; posterior area distinctly embossed, velured with chitinous asperities, interspersed with large round and elongate punctures, longitudinally striated, bearing several short setae near each lateral limit. Presternum of prothorax almost glabrous; eusternum with distinct semicircular anterior margin, beset with numerous setae in the central area; sternellum velured in the anterior area. Leg rudimentary, represented by slightly protuberant coxa bearing a few short setae. Spiracle round-oval, smaller posteriorly.

Ambulatory ampulla of abdomen provided dorsally with a median and two transverse impressions and ventrally with a single remarkably curved impression bordered with round and asperated tubercles. Pleural tubercle with two setae but without pit. Anal segment cylindrical, projecting, bilobed around the anus by a horizontal cleft, provided on the dorsal lobe with twelve and on the ventral lobe with twenty setae.

Notes on Life History and Habits.

The food plant of this borer, so far as observations go, is *Cinnamonum camphorum*. According to Dr. Maruyama, *Machius longifolia*

is also attacked by this pest. The imago appears in June and July and deposits eggs in the living trunk and branch, probably after gnawing egg incisions. The larva feeds for some time on the sappy portion, excavating irregular galleries under the bark, and works out long galleries in the direction of the major axis of the plant. Saw dusts are excluded from a small round hole to the exterior.

For the completion of life cycle this beetle seems to require two years or more, judging from the facts that only immature larvae of about 3 cm. in length were collected in March 27, 1930, while in February, 1928, a grown larva was collected by Dr. J. MARUYAMA in Ehime prefecture, and that on August 10, 1928, several grown larvae were collected in Tokyo.

The damage done is serious, and the larva devours a great deal of the cambial region.

Tribe MESOSINI.

Mesosa longipennis BATES.

(Pl. XVII, Figs. 14–15 and Text-fig. 13.)

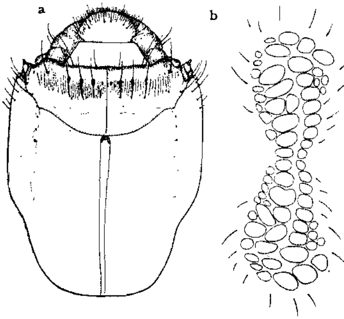
This species is commonly distributed in Hokkaido and Honshu, but nothing is known of its immature stages and life history. The following description is based on the material collected by the author in Tokyo and Chiba prefecture as well as on that kindly placed by Mr. H. YUASA and Dr. N. ISHIMORI in his hand.

MATURE LARVA.

Body white with a slight touch of yellow, testaceous in the prothorax, firm in the texture of integument, broadest and slightly depressed in the prothorax, slightly tapering backwards in the thorax, armed with a minute chitinous caudal spine, possessing very small anal segment which is slightly visible from the dorsal side, measuring in the largest specimen 37.1 mm. long by 6.2 mm. across at the prothorax.

Head capsule strongly castaneous in the entire emerged portion, not highly depressed, oval in cross-section, about 1.2 times as long as wide, with sides parallel anteriorly and suddenly constricted at the posterior one-third, and rounded at the posterior margin. Frons occupying about the anterior one-third of the head capsule, distinctly impressed with a median line and broadly curved and slightly notched frontal sutures, longitudinally striated and furnished anteriorly with four pairs of setigerous punctures which extends conspicuously backwards exclusive of the outer pair, smooth

in the posterior half, bearing two pairs of setae close to frontal sutures; epistoma with three pairs of long setae of which one pair is close to the median line and the rest are near the lateral limits. Subdorsal stripes of epicranial halves distinct, diverging backwards, beset anteriorly with a few short setae. Hypostoma slightly curved backwards at the anterior margin, beset with a pair of setae antero-medially; gula represented by a non-chitinized median impressed line; occipital foramen nearly circular. Ocellus one on each side, distinct. Antennal ring bisected by a frontal suture and highly raised at the margin; basal skin small, slightly emerged dorsally; first joint very short; second joint tapering distads, as long as wide; third joint elongate, cylindrical or slightly narrowed forwards, bearing four or five setigerous sensillae; accessory joint elongate-conical, a little shorter than the third. Labrum suboval, densely beset with golden coloured setae in the anterior half and on the lateral margins. Clypeus slightly excurved on the sides, glabrous. Mandible dark castaneous to black, about 1.5 times as long as its condylar width, acute at the apex, dentate at the dorsal angle; molar face with an oblique carina; outer face rather shiny, exhibiting a narrow longitudinal faintly depressed area in the anterior half, irregularly rugose and beset with two setae in the basal half. Lacinia conspicuously swollen anteriorly, shorter than two basal joints of maxillary palpus taken together; each joint of maxillary palpus longer than wide, conspicuously narrowed distads in the terminal joint; maxillary sclerite with a group of several setae at the boundary near the submentum; ligula straight at the anterior margin; mentum much shorter than half the width, bearing two long setae on each side; submentum without anterior boundary, beset with a pair of long setae; basal joint of labial palpus highly elongate, swollen anteriorly; terminal joint much larger than that of maxillary palpus.



Text-fig. 13. *Mesosa longipennis* BATES.
a Head of mature larva, dorsal aspect.
b Dorsal abdominal ampulla of the same.

Pronotum subrectangular, impressed anteriorly with a median line and in the posterior half with lateral sutures; anterior area testaceous, remarkably curved forwards and fringed with a transverse series of setae on the anterior margin, with a pair of setae at about the middle close to the median line; middle area fleshy, provided with several setae in the anterior and lateral portions, with faint vermicular rugae; posterior area fleshy, longitudinally striated, with a few setae anteriorly and laterally. Eusternum of prothorax fused with presternum and epipleurum, beset with numerous short setae. Metanotum with a single transverse impression which presents a loop medially and is bordered with large smooth tubercles of a round or oval shape. Leg small, two-jointed; coxa setose, rather protuberant; terminal joint slightly protuberant, bearing two setae. *Spiracle broadly oval, testaceous at the margin, smaller in the posterior* segments.

Ambulatory ampulla of abdomen furnished both dorsally and ventrally with a single transverse groove surrounded by numerous large fleshy tubercles. Epipleurum protuberant, bearing several long setae; pleural tubercle with two long setae but without pit. Ninth abdominal segment clothed with numerous setae, provided with a minute chitinous caudal spine. Anal segment with Y-shaped clefts, beset with about five setae on each lobe and six setae in the ventral area.

PUPA.

Creamy white, 18–25 mm. in length. Antenna forming a large single loop ventrally in the male, slightly shorter in the female; frons presenting a shallow mid-longitudinal groove, sparsely beset with long setae as in gena; clypeus with a mid-longitudinal ridge, beset on each side with two long setae; labrum convex above, provided with two transverse belts of dense setae at the middle and apex. Pronotum sparsely asperated with small setigerous and chitinous spines, broadly grooved along the median line; meso- and meta-notum asperated much as in the pronotum; scutellar groove distinct and broad in the metanotum. Tergite provided with numerous long, acute and curved setigerous spines; sternite with a few small setigerous spines; caudal segment with numerous stout spines of similar form along the posterior margin; the area anterior to the anus exhibiting two large contiguous tubercles in the female, trapezoidal and smooth in the male. First abdominal spiracle oblique, covered with wing pad.

NOTES ON LIFE HISTORY AND HABITS.

The food plants, so far as determined by the author, are *Pasania cuspidata*, *Ailanthus glandulosa*, *Cladrastis amurensis*, *Mallotus japonicus* and *Tilia cordata*. According to Mr. H. YUASA, *Quercus* sp. is also attacked by the present form.

The beetle appears extending from June to August and lays eggs in the branch and trunk of fresh dead trees. The larva, so far as observations go, feeds on the sapwood just beneath the bark and works out a long irregular gallery. It pupates in an oval pupal cell which is packed with fibrous shreds at the end of the gallery under the bark. However, some larvae were observed to bore into the heart of the decayed *Mallotus japonicus* and *Tilia cordata*.

The duration of life cycle is presumed to be one year, judging from the fact that the larvae collected in the same season are nearly in the same stage of growth.

Tribe SAPERDINI.

Paraglenea fortunei SAUNDERS.

(Pl. XVII, Fig. 16 and Text-fig. 14.)

Glenea chloromelas THOMSON.

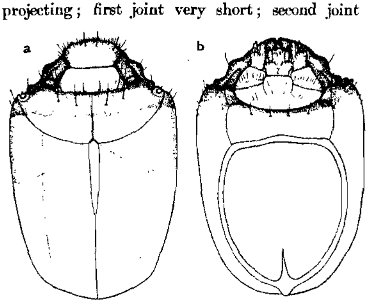
The present species has been recorded from Kyushu and North China, but nothing has hitherto been known of its immature stages. The material examined was kindly placed by Mr. S. KINOSHITA and Dr. I. KUWANA in his hand.

MATURE LARVA.

Body white, testaceous in the prothorax, rather thin in the texture of integument, sparsely clothed with long setae, elongate, tapering backwards from the prothorax to the end of body, measuring in the largest specimen 35.8 mm. in length and 5.1 mm in width at the prothorax.

Head capsule castaneous dorsally anterior to the middle of frons and ventrally in front of the occipital foramen, highly depressed, about 1.3 times as long as wide, broadly curved on the sides converging posteriorly into the slightly angulated posterior margin. Frons shorter than a quarter the length of head capsule, distinctly impressed with a median line and broadly curved frontal sutures, smooth on the surface, bearing three pairs

of long setae in the epistoma and also three pairs of long setae near the anterior margin. Subdorsal stripes in epicranial halves distinct. Hypostoma smooth, bearing four long setae in a transverse row; gula not defined. Ocellus absent. Antenna short, with closed ring; basal skin small but projecting; first joint very short; second joint conical, slightly elongate; accessory joint smaller than the third, furnished apically with a few minute processes. Labrum testaceous, suboval, setose anteriorly; clypeus trapezoidal, bearing a few short setae near each lateral limit. Mandible black but brown in the basal portion, about 1.5 times as long as its condylar width, dull at the apex and dorsal angle, faintly carinated on the molar face, beset with two setae on the outer face. Ligula large, rounded at the apex; mentum with a pair of long setae; submentum not separated from mentum, with a pair of short setae; basal joint of labial palpus elongate-cylindrical, about as long as the two basal joints of maxillary palpus taken together; terminal joint longer than that of maxillary palpus.



Text-fig. 14. *Paraglenea fortunei* SAUNDERS.
a Head of mature larva, dorsal aspect.
b Same, ventral aspect.

Pronotum impressed with a median line throughout the length and with lateral sutures in the posterior two-thirds which turn inwards anteriorly; anterior area testaceous, remarkably produced medially and beset with sparse numbers of long setae on the anterior margin; middle and posterior areas asperated with minute round chitinous tubercles which are flattened but raised posteriorly; on each side of the middle area is found a broad depression which is smooth and castaneous. Presternum of prothorax fleshy, with numerous setae; eusternum ill-defined at the anterior margin, setose anteriorly, asperated posteriorly; sternellum asperated anteriorly. Mesonotum asperated anteriorly. Leg rudimentary, two-joined; coxa scarcely elevated, bearing two setae; terminal joint cylindrical, beset with two setae. Spiracle small, elliptical, greyish brown at the margin, smaller in the posterior segments.

Ambulatory ampulla of abdomen asperated with minute chitinous tubercles which are pointed at the apex, furnished dorsally with two convex impressions meeting at each lateral limit and a median impression, ventrally with a single impression which is slightly angulated backwards. Epipleurum protuberant, beset with numerous long setae; pleural tubercle with two long setae but without pit. Anal segment highly projecting, trilobed by Y-shaped clefts, beset on each lobe with several setae.

Notes on Life History and Habits.

The present species is said to be highly noxious to ramie plantation. Its larvae were collected from the stem of the ramie, *Boehmeria nivea*, in the prefecture of Nagasaki.

EXPLANATION OF PLATE XVII.

The asterisk marks the photographs mounted through the courtesy of Mr. S. Kinoshita.

1. Larva of *Megopis sinica* White, lateral aspect.
2. Larva of *Prionus insularis* Motchulsky, dorsal aspect.
3. Larva of *Psephactus remiger* Harold, lateral aspect.
4. Pupa of *Psephactus remiger* Harold, ventral aspect.
5. Larva of *Mallambyx raddei* Blessig, dorsal aspect.
6. Larva of *Monochammus grandis* Waterhouse, dorsal aspect.
7. Pupa of *Monochammus grandis* Waterhouse, ventral aspect.
8. Pupa of *Monochammus sartor* Fabricius, ventral aspect.
9. Larva of *Monochammus tesserula* White, dorsal aspect.
10. Larva of *Dihammus luxuriosus* Bates, dorsal aspect.
11. *Pupa of *Dihammus luxuriosus* Bates in the pupal chamber of *Aralia chordata*.
12. Larva of *Melanauster chinensis* Förster, dorsal aspect.
13. Larva of *Eupromus ruber* Dalman, dorsal aspect.
14. Larva of *Mesosa longipennis* Bates, dorsal aspect.
15. Pupa of *Mesosa longipennis* Bates, ventral aspect.
16. Larva of *Paraglenea fortunei* Saunders, dorsal aspect.
17. *Cross section of the trunk of *Castanea sativa* infested by *Mallambyx raddei*.

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The Oestrous Cycle and Ovulation in the Rabbit.

By

Seigo Shibata.

With Plates XVIII–XX and four Text-figures.

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Introduction.

Among rabbit-breeders it is stated that there is in the female no authentic indication of oestrum other than her desire to mate and that other signs, such as the degree of enlargement or inflamed appearance of the vulva, jumping one another, etc., are no credible guide for the female. But to this sign of heat, her willingness to copulate, there are sometimes exceptions, especially when the doe is in pregnancy, in lactation, or during the immature period. Moreover, such cases often occur as that a normal adult female having refused one buck will accept another with readiness. The act of copulation, therefore, is a subsidiary but not completely reliable criterion for the determination of oestrum in this animal.

In order to determine more precisely the oestrous cycle in the rabbit several workers pay attention to the microscopic structure of the genital organs. In spite of their elaborate investigations the sexual cycle in the living state is not made out clearly.

From mere breeding experience some men mention that the heat of the rabbit occurs every two weeks, every three weeks, or once a month, while others put forward the view that the doe has no regular oestrous cycle and stands in the state of oestrum for a long duration.

Now, following the analytical method of the vaginal smear, which was originally introduced by STOCKARD and PAPANICOLOU (1917, 1919) in the guinea-pig and thoroughly observed by LONG and EVANS (1922) in the rat, many workers point out in the most domestic mammals and even in human beings the occurrence of a close relation between the process of follicular growth in the ovary and the cellular composition of the vaginal discharge. With the rabbit, however, SNYDER (1926) and KUNDE and PROUD (1929) state that, although the smear from the vaginal lumen shows marked cellular changes, no cyclic recurrence of oestrum can be demonstrated, and that as coition occurs readily in this animal either when the smear contains a number of leucocytes or when it is entirely devoid of them, the vaginal smear method is of no use in the prediction of the oestrous period.

In the majority of mammals the rupture of the mature ovarian follicle takes place spontaneously, and the formation and persistency of the corpus luteum regulates and inhibits the ripening of the follicle, with the result that in all parts of the genital organs there follow alternately the oestrous and dioestrous conditions. As ovulation is usually dependent in the rabbit on sexual intercourse, without which no corpus luteum develops, the rhythm of oestrum may possibly be obscure and irregular to a certain degree. But so long as some series of follicles successively develop in the ovary, the doe must be at oestrum when the follicles ripen and at dioestrum when they degenerate, *i.e.*, the oestrous cycle should still exist in the rabbit however unclear its indication may be.

Since July, 1925, the author has undertaken several series of experiments with respect to the oestrum and ovulation in the rabbit, for the purpose of determining the cyclic change of the oestrous phenomena by means of the vaginal smear. The results are partly coincident with those obtained by SNYDER and others, but in the main point, particularly as regards the determination of the oestrous cycle, they are quite different from the conclusions reached by other observers.

The present work was carried out at the Animal Breeding Experiment Laboratory of the Tokyo Imperial University largely with the subvention from the Imperial Academy, for which the author wishes to express his heartiest thanks.

Material and Methods.

The material used in the present work consists of more than 200

females and some males, which were partly purchased from local rabbit-breeders and partly bred in the laboratory. The majority belong to the "American cross-bred" white rabbit, the commest breed in Japan. Each animal was kept in a separate cage made by the author himself.

The colonies were fed with bean-curd refuse, wheat, oat, and bran with hay and vegetables during the winter and with freshly-cut grass during the warm season.

The vaginal smears subject to the microscopic observation were made from the substances that happened to be present in the inner part of the vaginal lumen, 7–10 cm. in depth. They were fixed in methyl-alcohol and stained with the diluted solution of ROMANOWSKY-GIEMSA'S "Azur-Eosin-Methylenblau".

For the histological investigation the reproductive glands were fixed in ZENKER'S fluid and the uterus and vagina in ALLEN'S modification of BOUIN'S fluid. Most serial sections, 5–12 μ in thickness, were stained with DELAFIED'S haematoxylin. In addition to these vaginal smear and histological methods several series of extirpation, injection and transplantation experiments were carried out. The details will be mentioned in each of the following items.

Results.

1. THE OESTROUS CYCLE.

GENERAL OBSERVATION OF THE GENITAL TRACT.

In the rabbit the uterus is of the bicornis type, the horns being nearly equal in length and opening separately into the vagina. Notwithstanding the absence of the corpus uteri and cervix uteri, the orifice of each horn is commonly named cervix. The uterus is made up of 5 layers: (i) the serous membrane, (ii) the longitudinal muscle layer, (iii) the circular muscle layer, (iv) the mucosa composed of the connective tissue with a number of blood vessels, and (v) the epithelium of ciliated cuboidal cells. The mucosa, though varying from 4 to 8 in number, generally forms 6 folds, *viz.*, 2 placental folds, the largest, containing numerous blood vessels but a few tubular glands, and 2 periplacental and 2 obplacental folds presenting numerous glands but a few blood vessels. The uterine glands lined with a thin epithelial layer of cuboidal cells open out into the cavity of the uterus. They are found even near the junction point of the uterine and the vaginal mucosa. There is no distinct boundary in the structure of the

epithelium between the horns and the vagina. The following are the measurements based upon 10 normal adults.

	Average	Maximum	Minimum
Body-weight	2757 gr.	3000 gr.	2550 gr.
Horn-length	9.90 cm.	14.00 cm.	7.50 cm.
Horn-breadth	0.69 „	0.90 „	0.55 „
Lumen-diameter	0.62 „	0.85 „	0.50 „
Epithelium-thickness...	17 μ	20 μ	12 μ
Muscosa-height	1718 „	2750 „	1000 „
Circular layer-thickness	510 „	700 „	350 „
Longitudinal layer-thickness	480 „	600 „	350 „

The vagina is a broad passage from the uterus to the exterior. Its mucosa is thrown up into a number of folds, and is lined with a simple or a very *slightly* stratified epithelium, much as in the uterine epithelium. Externally the mucosa is surrounded by the longitudinal and circular muscle layers. The urinary bladder has its orifice at the inner end of the vestibulum.

Sorts of cells in the vaginal smear.

The cells contained in the vaginal fluid are more similar in kind to those in the fluid of the guinea-pig than in that of the rat, although the mode of their appearance differs considerably.

The cells found in the vaginal smear of the rabbit can be roughly classified into 3 groups or 5 types.

Group I. Cornified epithelial cells, the amount of which is indicated with broken-lines in the accompanying figures, consisting of 2 types: (A) cells of a squamous type, large in size (50–80 μ), with small pycnotic nuclei (7–16 μ), having the protoplasma reticular in structure and weakly stainable, and (B) cells completely cornified, large in size (50–80 μ), non-nucleated, most weakly stainable.

Group II. Normal epithelial cells, the amount of which is indicated

with full-lines in the figures, consisting of 2 types: (C) cells of a moderately healthy appearance, medium in size (20–40 μ), with fairly well stainable nuclei (10–15 μ), and (D) cells of a more healthy appearance, round in shape and very small in size (10–20 μ), with deeply stainable and well defined nuclei (5–15 μ).

Group III. (E) Leucocytes, the amount of which is indicated with chain-lines in the figures and is twice as much as the other groups, the majority being polynuclear-neutrophile leucocytes.

Histological examination proves that all these cells are derived from the inner portion of the vaginal epithelium, especially from the cervical folds, in company with a few from the uterus.

In the rabbit the cornified epithelial cells of the two types A and B are rarely found in the smear, though they do not independently appear in abundance at any reproductive period. On the other hand, the appearance of the leucocytes is not only confined to the normal oestrous period but some numbers of them are oftentimes observed at the times of pregnancy and lactation, or even before puberty. Generally the leucocytes appear in much abundance in the warm season than in the cold. Particularly the vaginal fluid becomes crowded with enormous numbers of the leucocytes after coition and parturition. It is therefore almost impossible to determine the sexual cycle in the rabbit by observing the scanty existence of the cornified epithelial cells and the irregular occurrence of the leucocytes. The failure in the determination of the oestrous cycle by SNYDER and others seems to have been caused mainly by the overestimation of the appearance of the leucocytes. The author's attention, as will be seen later, has been focused upon the behaviour of the healthy nucleated epithelial cells belonging to the types C and D, the recurrence of which is considerably regular and is uniformly correlated with the follicular activity.

WITH THE IMMATURE RABBIT.

Making use of 3 immature rabbits (Nos. 158, 159, and 160), all 90-days-old, and a young but mature doe (No. 156), 180-days-old, the vaginal smear was examined twice a day (8–9 A.M., 3–4 P.M.) for the duration of 18 days. On the 19th day they were killed and subject to examination.

Histologically, the 3 immature females showed almost the same appearance in the structure of the ovary, uterus and vagina. The muscle layers of the uterus and vagina were very thin (50–80 μ), and the mucous

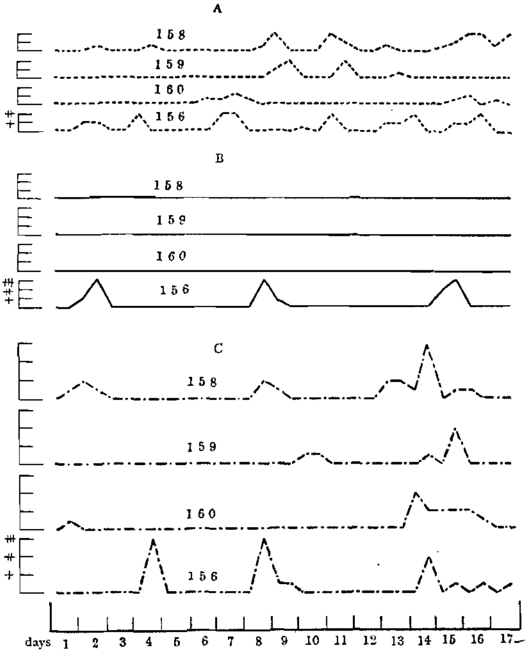
folds were very small and low and measured 200–250 μ in height in the uterus. The uterine glands had yet scarcely developed in the animals, though one of them (No. 158) presented a few masses of glandular cells. Neither vascular congestion nor haemorrhage was noticed in the uterine stroma.

No. of Rabbit	Body-weight (gr.)	Ovary-length (cm.)		Ovary-breadth (cm.)		Ovary-weight (gr.)	
		L.	R.	L.	R.	L.	R.
158	1170	1.55	1.45	0.40	0.45	0.093	0.088
159	1140	1.55	1.25	0.40	0.45	0.079	0.066
160	990	1.30	1.15	0.45	0.45	0.088	0.079
Control	2000	1.90	1.60	0.45	0.45	0.108	0.124

The ovary was bright and smooth in the surface view and presented no evidently developed follicle. In serial sections, however, large numbers of primitive and developing follicles were demonstrated. In Nos. 159 and 160 they were small and measured about 300 μ in diameter, while in No. 158 were found 2 medium-sized follicles of about 500 μ in the left ovary and 4 of similar size in the right.

In the control (No. 156) the vaginal lumen was very narrow, and the folds covered with a thin epithelium (5–13 μ in thickness) were not yet fully developed. Each of the uterine cavities was also narrow and flattened, and the folds covered with a thin epithelial layer (15–20 μ) were more or less enlarged (1000 μ in height). The uterine stroma was densely composed of the connective tissue with a number of slightly congested blood vessels. There existed many uterine glands, though small in size, in the thin portion of the stroma. The interior circular muscle layer was 450 μ in thickness, and the exterior longitudinal layer 400 μ. Each ovary contained a large number of primitive and developing follicles, of which 3 fairly-enlarged follicles in the left ovary and 2 in the right measured about 700 μ in diameter. The growth of interstitial glands was very poor.

As indicated in Text-fig. 1, Nos. 158, 159 and 160 showed an irregular occurrence of the cornified epithelial cells and leucocytes, but no trace of the nucleated epithelial cells in the vaginal smear. On the contrary, No. 156 presented not only the cornified cells and leucocytes, but also evidently a lot of nucleated epithelial cells of the types C and D thrice during the experiment. These facts appear to indicate that there is



Text-fig. 1. Diagrams showing the occurrence of the cornified cells (A), the nucleated epithelial cells (B) and the leucocytes (C) in the vaginal smear of 3 immature and 1 mature rabbits.

a striking coincidence between the occurrences of the nucleated epithelial cells in the vaginal smear as well as in the reproductive organs.

Relation to copulation.

Copulation in the doe takes place when the vaginal smear presents any kind of cellular changes as well as when it contains no cellular substance.

But at the time when the vaginal fluid has a maximal rate of the leucocytes the doe is liable to refuse the buck, and even if she willingly accepts him the percentage of fertilization is very low. From the general point of view, therefore, it is also true in this animal that the more the leucocytes occur in the vaginal cavity at coition, the more injurious effect they exert upon the spermatozoa, although not so long after every coition the emigration of the leucocytes through the uterine and vaginal walls is usually accelerated. The following table indicates the relation between the vaginal smears and the results of copulation.

(a) Refused to copulate.				(b) Copulated but infertile.				(c) Followed by Pregnancy.			
No. of Doe.	Group of Cells. I.	II.	III.	No. of Doe.	Group of Cells. I.	II.	III.	No. of Doe.	Group of Cells. I.	II.	III.
15.	—	—	—	75.	—	—	—	56.	—	—	+
16.	—	—	—	8.	++	+++	—	16.	+	—	—
15.	—	—	—	63.	—	+++	—	51.	+	—	—
18.	—	—	—	9.	+	++	+	60.	+	+	—
56.	++	+++	—	61.	—	+++	+++	14.	+	+	—
18.	—	+	++	6.	+	—	+++	18.	+	++	—
36.	—	+	++	5.	+	—	++	77.	—	+++	—
17.	—	—	+++	7.	—	—	++	70.	—	+++	—
53.	—	—	++	79.	—	—	+++	9.	—	+++	+
51.	—	—	+	4.	—	—	+	78.	+	—	++

Remarks: (i) The smears were collected a few minutes before each trial for copulation.
(ii) + = a small number of cells.
++ = a tolerable number of cells.
+++ = a large number of cells.
(iii) The above symbols are commonly used in the text-figures of this paper.

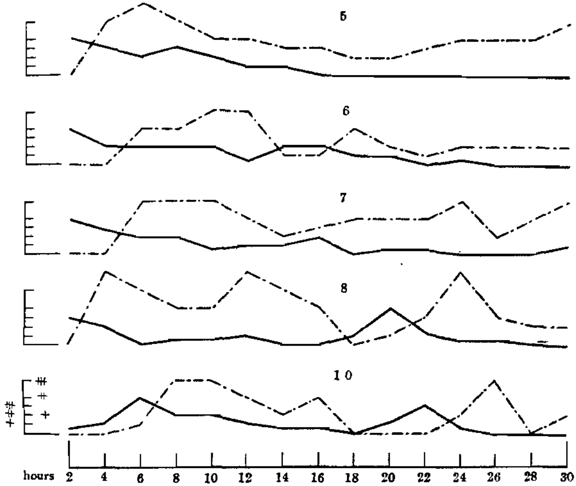
As may be evident from this table the absence of the leucocytes in the vaginal lumen at the time of coition appears to be the most favourable condition for inducing successful fecundation.

During 30 hours after copulation.

A great deal of attention has been paid by some observers to the vaginal plug of the rodent. After every coition the spermatic fluid of the rabbit coagulates to form the vaginal plug which prevents the outflow of

the sperm, although it is not so rigid and typical as in other species of the rodent. Setting aside here the use, structure, chemical composition, etc. of this plug, the vaginal smear in 5 rabbits (Nos. 5, 6, 7, 8, and 10) was investigated, its cellular changes being cited in Text-fig. 2. As for the cornified cells, they are left out of consideration.

The smear was gathered every 2 hours for 30 hours after every coition. In each case the nucleated epithelial cells suddenly appeared in abundance from 2 to 8 hours after copulation. (This sort of cells can be found in the normal sexual cycle, but after copulation they are most abundant and distinct. Sometimes a number of the nucleated epithelial cells may reappear during 20–22 hours after copulation.) Myriads of the leucocytes occurred between 4 and 12 hours after coition and lasted in an irregular state. In general they were most plentiful at the time of ovulation, about 10 hours after sexual intercourse.

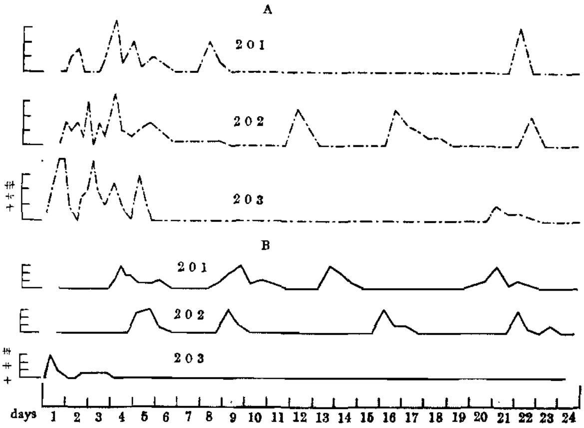


Text-fig. 2. Diagrams showing the occurrence of the nucleated epithelial cells (full-line) and the leucocytes (chain-line) in the vaginal smear, during 30 hours after copulation.

After sterile copulation.

It is properly recognized from the histological point of view that after infertile coition the corpus luteum is usually formed in the rabbit's ovary, and that the corpus luteum develops to its largest size 7–10 days later, and on the 16th day or so of pseudo-pregnancy the cells of the theca interna notably proliferate inwards, while the luteal cells become less and less numerous, finally disappearing between the 25th and the 30th day. It does not need to explain that with the growth of the corpus luteum the uterine mucosa begins to show marked hypertrophy, and with its degeneration the uterus gradually begins to return to the initial state. Notwithstanding these notable changes of the ovary and uterus, the vaginal mucosa, as a rule, rather decreases in height; and its epithelium shows almost no sign of desquamation and remains in a prolonged dioestrous feature during pseudo-pregnancy.

In this study, 2 sterile bucks, previously proved to be efficient breed-



Text-fig. 3. Diagrams showing the occurrence of the leucocytes (A) and the nucleated epithelial cells (B) in the vaginal smear in the cases of 2 infertile copulations, and 1 pseudo-pregnancy.

ing animals, were prepared by removing small pieces of the vas deferens on each side of the body. 5–7 days after operation, they were mated with 2 females (Nos. 201 and 203) respectively. At the same time a weak young doe (No. 202), 6-months-old, was allowed to copulate with a non-vasectomized buck. The vaginal smears were collected from these 3 females 4 times a day (8 and 11 A.M., 2 and 5 P.M.) for the first 4 days and twice a day (8–9 A.M., 4–5 P.M.) for the following 20 days.

In each animal for 5–6 days following coition there were found in the vaginal lumen myriads of the leucocytes, which later decreased in number, though they recurred somewhat abundantly at irregular intervals. *As regards the nucleated epithelial cells, they appeared more or less periodically* both in Nos. 201 and 202, while in No. 203 they were never present except for the first 3 days.

On dissection it was found that only in No. 203 ovulation had taken place in company with the formation of the corpora lutea, but that in Nos. 201 and 202 none of the follicles had ruptured. Very few as examples are, the difference in the vaginal condition between the pseudo-pregnancy and the normal was clearly distinguished by paying attention to the occurrence of the nucleated epithelial cells.

In pregnancy.

The changes occurring in the uterus and vagina during pregnancy are similar in general features to those taking place during pseudo-pregnancy. The vaginal mucosa at the time of gestation is not only so intensively changed, but it rather maintains the feature of prolonged dioestrum, although in the later stage many vacuolated epithelial cells appear in the surface layer of the cervical folds. In the uterus, at the beginning of pregnancy, the degenerated epithelial cells, mostly from the obplacental folds, begin to exfoliate into the lumen. After the middle period, when a part of cells in the corpora lutea as well as in the placenta begins to regress, cells of the old uterine mucosa-membrane show still more degeneration, although remaining in a few numbers in the cavity even till the end of pregnancy.

By the vaginal smear method 31 pregnant rabbits were examined in order to know whether the oestrous phenomena occur during gestation. The hypertrophied atypical cells desquamated from the cervix and uterine folds were found at irregular intervals in the vaginal fluid. The leuco-

cytes were not alone found in the first stage of gestation, but in the majority of cases they were observed irregularly throughout the pregnant period. The extensive exfoliation of the nucleated epithelial cells of the types C and D began immediately after each copulation and lasted for 1–5 days. Thence during the mid-pregnancy of about 20 days the oestrum-like feature, which was presumed by the occurrence of numerous nucleated epithelial cells, was recognized 1–3 times in each of 15 individuals, but not in other 16 animals. Moreover, during the last 1–5 days of pregnancy, cells of the type C, sometimes mixed with vacuolated gigantic cells, recurred vividly in the smears from 17 animals. The epithelial cells and leucocytes had a tendency to flow out more frequently and more abundantly in the warm season than in the cold.

These facts stand in relation to the fact that in some rabbits copulation often takes place during pregnancy. Following the second or the third coition during gestation the ripening of the follicles will be accelerated, yet ovulation from these follicles is inhibited by the effect of the newly formed corpora lutea.

After parturition.

5 rabbits (Nos. 12, 13, 31, 32 and 33), from which litters had been removed soon after parturition, were killed at varying periods from 1 to 2 weeks. In none of their ovaries was found any newly ruptured follicle or any fresh corpus luteum. Other 5 does (Nos. 11, 19, 20, 23 and 24) were mated within 1–3 days after each delivery whether they were suckling or not. In 3 of them, killed on the 5th day after copulation, were discovered newly developed corpora lutea. Except for a few days just after parturition, any doe tried did not accept the buck so long as the suckling continued; and the structure of the vagina during lactation showed only the persistence of dioestrous condition, the characteristics of which were not so distinct as in other rodents.

The vaginal smears were collected at 3 hours' intervals for 72 hours following parturition from 2 mother does without litters, and from 1 with litter. A shower of leucocytes with haemorrhagic fluid was a marked occurrence immediately after delivery. The leucocytes continued to be present for a long duration. Numerous nucleated epithelial cells began to appear at varying periods from 15 to 39 hours after parturition. They reached the maximal amount at 33–36, 42–48, and 18–24 hours respectively, when the buck would easily be accepted.

Another microscopic examination of the vaginal smears, obtained from 5 suckling mothers (Nos. 2, 16, 5, 59 and 35) daily for 23 days, indicated that during 13–21 days or more merely some leucocytes and scattered atypical epithelial cells were sometimes present in the lumen of the vagina, *i.e.*, lactation presented a tendency to inhibit the maturity of the follicles also in the rabbit.

Text-fig. 4. Diagrams showing the occurrence of the nucleated epithelial cells (full-line) and the leucocytes (chain-line) in the vaginal smear, during 72 hours after parturition.

In 7 does (Nos. 1, 3, 7, 10, 8, 14 and 69), from which all youngs were removed at delivery, the recurrence of oestrum took place 3–12 days later.

After ovariotomy.

From each of 7 adult rabbits (Nos. 163, 164, 165, 166, 170, 171 and 172) the ovaries on both sides were carefully removed, making two *incisions just posterior to the last ribs. Beginning on the* 11th day after operation, the vaginal smears were gathered 3 times a day (8–9 A.M., 12–1 and 4–5 P.M.) for 80 days. During the experiments, No. 170 died, Nos. 164 and 165 were killed, and No. 172 became pregnant and delivered 5 healthy youngs on the 86th day after spaying.

On antopsy, practised on the 90th day, a minute piece of broken ovary (0.013 gr.) was discovered on the left side of the abdomen in No. 166, and two fine pieces of ovaries (0.017 gr. and 0.057 gr. each) were found

in No. 172. These fragments were not the regenerated but merely residual portions of the ovaries. Each contained atretic follicles of various sizes in addition to the corpora haemorrhagica or the corpora lutea, without presenting any healthy developed or primitive follicle. Completely ovariotomzied females were 5 in all. One of them (No. 171) copulated several times, while the other 4 refused the buck.

It was observed from the vaginal smears that in No. 165 the heat ceased throughout, while in the other 4 oestrum-like indication, which was presumed from the existence of abundant nucleated cells, occurred 3–7 times, particularly during the 4th–7th weeks after operation. Nos. 166 and 172, which possessed the remaining ovaries, mated frequently, and the vaginal smears from them showed striking oestrous symptoms. The details were placed on record in the previous paper (1930).

It is of some interest that even in the perfectly spayed rabbits oestrum-like changes were recognized in the vaginal smear. But if observation is continued for a few weeks, setting aside this unexpected occurrence for a time, the distinction between complete and incomplete ovariotomized rabbits can be demonstrated by means of the vaginal smear.

Normal oestrous cycle.

At every stage of the cellular changes appearing in the vaginal smear, 20 normal adult rabbits were killed, and their genital organs were histologically examined. In the serial sections of the uterus and vagina there were demonstrated various parts of the epithelium going to desquamate, sending out leucocytes, or retaining quite healthy feature. Consequently it seems that all portions of the epithelium do not necessarily change at the same time, but the renewal process is very slow or partial at every oestrum. Considerable difficulty is naturally experienced in the histological investigation of a close relation between the changes of the vaginal or uterine epithelium and those of the ovarian follicle. If a general comparison is, however, made with many females, the development of the follicle, in association with the animation of the genital tract, may be recognizable.

Some females (Nos. 9, 14, etc.) which had no indication of cellular changes in the vaginal smear for a long period exhibited only small-sized follicles (under 200 μ in diameter) in their ovaries, and their uteri and vaginae were short and thin. Some others, in which more or less regular

oestrous changes were observed in the vaginal smear, had either medium-sized follicles (300–700 μ) with small but rather round genital tracts (Nos. 105, 111, etc.), or well developed healthy follicles (800–1200 μ) with rather long and thick uteri and vaginae (Nos. 110, 104, etc.).

With 23 normal does the vaginal smear was traced for 365 days, 10–14 of them being used in turn twice a day (9–10 A.M., 3–4 P.M.). Oestrum was determined by the distinct occurrence of the nucleated epithelial cells in the smear. When an oestrous state lasted for several days, only the first day was recorded. The results were published by the author in 1927 in the form of a preliminary report, the main points being briefly cited here.

Oestrous Cycle in Days. / Month.	3	4	5	6	7	8	9	10	11	12	Total	Average	Prolonged Cycle in Days
Aug.	1	8	5	5	5	3					27	5.52	
Sept.	1	4	6	12	13	3	4				43	6.33	
Oct.		3	8	16	11	3	1	2			44	6.32	
Nov.		2	1	11	15	9	6	5	1	1	51	7.66	
Dec.			1	6	6	5	6	1	6	1	32	8.28	13, 16, 16, 16, 17, 17, 18.
Jan.			4	12	13	6	1	5	4		45	7.42	14, 14, 15.
Feb.				7	7	3	1	0	1		19	7.11	13, 13.
Mar.			3	8	4	4	1	1			21	6.76	
Apr.			3	9	13	3	1				29	6.76	
May		1	5	15	9	5	3	1			39	6.64	
June		1	6	15	15	5	1	1			44	6.55	
July		2	6	13	12	5	1				39	6.38	
Total	2	21	48	129	123	54	26	16	12	2	433	6.81	12 Cases

As is shown in this table, the duration of the oestrous cycle in the rabbit is 6.81 days on an average, ranging from 3 to 12 days, with the exception of 12 prolonged cases. Copulation takes place not only at the maximal oestrum, when a series of follicles is most developed, but also at the proestrous or often even at the anoestrous period. Moreover, as

the sexual cycle is thus very short, it is apt to be considered that the rabbit is always in a state of oestrum, although the rate of fertilization necessarily differs to a certain degree according to the vaginal conditions at the time of copulation.

2. The Cause of Ovulation.

The causes recorded by many authorities as inducing ovulation in the rabbit may be classified as follows:—(i) Spontaneous occurrence just after parturition, (ii) copulation with a normal or vasectomized buck, (iii) artificial insemination, though very rare, (iv) the courtship of a male or another female rabbit, (v) rubbing the vulva, (vi) the injection of the female sexual hormones, such as extracts or emulsions from the placenta, aciniform blood-mole, hypophysis, or urine of a pregnant woman. The cause of the stimulation processes may also be divided into two classes, *viz.*, *(A) the psychic mechanism and (B) the humoral mechanism. To the former* iv and v evidently belong and to the latter vi belongs. i, ii and iii are considered by some authors to come under A or by some others under B.

As the author's previous experiments proved that just after parturition no ovarian follicle is broken spontaneously in any rabbit, and that by the courtship with other individuals or by rubbing the vulva the maturity of follicles is not accelerated to any noticeable degree, but that, though only at a low percentage, the artificial insemination at the oestrous period can induce pregnancy, he came to hold the opinion that there should be some humoral substances to initiate the rupture of follicles in the rabbit's ovary. *This consideration has been intensified by the following experiments.* The results, with the exception of one case, were announced *viva voce* in April, 1928 and 1929, each at the annual meeting of the Scientific Agricultural Society held in Tokyo.

Removal of the genital tracts and injection of the anaesthetic.

a) In 12 rabbits the oviducts and uteri were removed, and 5–8 days after extirpation, each doe was placed twice with the buck, copulation following at every trial. At the time of autopsy after 12 days, fresh corpora lutea were found in the ovaries of 7 animals, in addition to one blood-follicle in the right ovary of No. 169. In No. 186, the lower half of the right ovary was oedematously swollen.

No. of Doe	Body-weight (gr.)	Ovary-weight (gr.)		Number of Corpora Lutea	
		L.	R.	L.	R.
167	2365	0.165	0.185	0	0
168	2420	0.185	0.275	2	5
169	1940	0.197	0.256	0	0
180	2500	0.141	0.215	2	5
181	2200	0.100	0.109	0	0
183	2230	0.192	0.228	1	3
185	2500	0.158	0.173	1	2
186	2530	0.325	*1.935	7	2
187	2650	0,242	0.272	3	5
188	2835	0.340	0.294	4	5
189	2770	0.147	0.161	0	0
190	2615	0.165	0.170	0	0

*= Ovarium oedema

b). Subsequently, in 7 rabbits the lower one-third of the uteri and the inner portion of the vagina, with the exception of the part between the external orifice and the neck of the bladder, were carefully removed. 17–19 days after operation copulation was carried out twice in each animal. 3–4 days later, at post-mortem, fresh corpora lutea were found in 2 does. In any case was observed neither blood-follicle nor oedema.

No. of Doe	Body-weight (gr.)	Ovary-weight (gr.)		Number of Corpora Lutea	
		L.	R.	L.	R.
191	3500	0.427	0.413	6	4
192	2280	0.115	0.093	0	0
193	2175	0.164	0.153	0	0
194	1745	0.107	0.103	0	0
195	1900	0.173	0.180	0	0
196	2020	0.128	0.104	0	0
197	3275	0.348	0.317	7	2

c). With a solution containing 0.3 gr. of cocaine hydrochloride and 5 drops of adrenalin chloride in 10 cc. of distilled water. In each of 10 female rabbits 0.5 cc. of this solution was injected subcutaneously on both sides of the vulva, and to make sure its effect it was also applied as an ointment to the lining membrane of the vagina. 10 minutes later every doe met readily with the buck. At the time of autopsy after 3 days, fresh corpora lutea were demonstrated in the ovaries of 6 animals. The left ovary of No. 209 was oedematously swollen as large as the right ovary of No. 186.

No. of Doe	Body-weight (gr.)	Ovary-weight (gr.)		Number of Corpora Lutea	
		L.	R.	L.	R.
206	2785	0.437	0.407	6	4
207	3080	0.235	0.293	4	6
208	2830	0.110	0.102	0	0
209	3085	*1.575	0.377	6	4
210	2745	0.128	0.205	3	7
211	3055	0.157	0.154	4	1
212	2800	0.098	0.091	0	0
213	3040	0.220	0.262	0	0
214	2705	0.106	0.107	0	0
215	2680	0.438	0.475	4	5

*=Ovarium oedema.

The percentage of ovulation was (a) 58.3, (b) 28.6, and (c) 60.0 in each experiment. This low percentage in the second case (b) seems to be due to the reduction of the absorptive surface of the seminal fluid, as the inner portion of the vagina was removed.

Judging from the results obtained from these 3 series of experiments, the cause of provoking ovulation in the rabbit's ovary is mainly ascribed to some humoral substance or substances rather than the excitement of the nervous system.

Injection of testicular hormone.

In 1927 and 1928, the author with Hirabayashi and Matsufuji

initiated ovulation artificially in 4 of 30 female rabbits by injecting the "Spermatin" made from the testicular and prostatic extracts, subcutaneously with 0.5 cc. dosage per head every other day 5–15 times. Moreover, the author practised further experiments injecting the emulsion of the male genital organs.

a) At first, 2 testes and prostates of a pig were cut into pieces, crushed in the mortar, and well mixed with 1 litre of the physiological salt solution. 2 cc. of this fluid were injected subcutaneously 1–2 times a day into 6 does which were killed and examined on the following day. All the uteri were found strongly congested, and several large prominent, fresh blood-follicles were demonstrated in 3 ovaries, as if they appeared at the time of ovulation.

b) Next, other 5 does were injected with the emulsion of the male rabbit's genital organs. At every trial 20 cc. of the physiological salt solution well mixed with two testes and prostates was newly prepared, and 1 cc. (per head a day) of this fluid were used every other day 4 times in all. In one of them (No. 182) there appeared prominent newly formed blood-follicles.

(a)			(b)		
No. of Doe	Number of Large Follicles		No. of Doe	Number of Large Follicles	
	L.	R.		L.	R.
162	6	3	178	4	4
173	5	7	179	2	8
174	2	5+2C.h.	180	6	6
175	3+3C.h.	8+4C.h.	182	4+2C.h.	4+4C.h.
176	5	6	183	6	5
177	3	2			

C.h. = Corpora haemorrhagica.

In these two series of experiments a number of well developed follicles was somewhat larger than that in the normal does. It would seem that some part of these follicles developed promptly by the stimulative effect of the male sexual hormone. As regards the corpora haemorrhagica the

capillaries of the follicles ruptured inwardly but did not ovulate, for the stimulus was not presumably so great.

TESTIS-TRANSPLANTATION.

In order to observe the effect of the male sexual hormone upon the ovary, the testes were removed from the young rabbits and were cut into two parts, one or two halves of these testes being implanted respectively into the wounded bleeding lips of the liver in 7 females. As controls the ovaries from 6 adult females were implanted into each liver of the other 6 females. One month after operation, the animals were killed, and the ovaries were examined microscopically.

	No. of Host	Body-weight (gr.)	Number of Follicles		Uterus
			Large sized	Medium sized	
Testis transplanted	117	2360	13	17	Medium
	118	1435	6	11	"
	119	2460	8	19	Large, but flattened
	120	1960	0	18	Small, but round
	122	1775	0	17	Medium
	124	1490	3	31	Smallest, like oviduct
	125	1815	8	23	Small
Ovaries transplanted	74	1540	5	5	Medium
	76	2065	2	6	Small
	81	1630	0	6	"
	84	1770	4	15	Rather large
	88	1570	0	15	Small
	90	2115	7	6	Small, but round

The implanted testis of No. 125 was extremely well adhered to the liver tissue. The tunica albuginea was exceedingly hypertrophied, as compared with that of anormal young testis (some 100 μ in thickness), and measured 250 μ in thickness. The newly increased connective tissue

stretched outwards into the liver substance from 4 corners, each projection being 585, 1825, 2080, and 2275 μ in length. The seminiferous tubules were not so much changed in gross appearance from those of a mormal rabbit, each tubule being 109-120 μ in diameter. In some of them, however, there being no kind of typical generative cells, were observed merely a few SELTORI's cells and a few irregularly distributed round gigantic cells, probably derived from generative cells, and most of the tubules were found still more degenerated. The tunica albuginea of other 6 implanted testes was more hypertrophied and 400–500 μ in thickness. The connective tissue was found invaded inwards. The seminiferous tubules became atrophied to the utmost and measured 50–90 μ in diameter, each tubule being filled only with non-constructed masses of protein. The interstitial portion between the tubnles was loosely replenished with many young mesenchyme cells, myriads of leucocytes, some lymphocytes, and metamorphosed abnormal cellular substances. In the hosts' ovaries, the number of the developed follicles was larger to some degree than that of the ovaries-transplanted. But in each ovary of the testis-transplanted, all large follicles (above 800 μ) and about two-thirds of medium-sized follicles (400–800 μ) were in the atretic condition, while most of the small follicles, 100–200 μ in diameter and 10–25 in number, remained healthy and seemed to promise the recovery of the whole follicular system for a short time.

Next, as for the transplanted ovaries of 5 does (Nos. 74, 76, 84, 88, and 90), they were surrounded by the connective tissue and thoroughly degenerated. No follicle and germinal epithelium were found in them. The implanted ovaries of No. 81, though also surrounded by the connective tissue, had considerably well-preserved germinal epithelium, and were devoid of healthy follicle. In each ovary of the hosts some atretic follicles of course existed, but the number of them was not so large as that of the testis-transplanted. *It was common in these series of experiments that the* initial process of atresia in the large and medium-sized follicles consisted in the degeneration of the granulosa cells, and in the small follicles the ovum was usually more markedly affected than the granulosa.

HYPOPHYSIS-TRANSPLANTATION.

As it has been studied by some authorities that in the rabbit ovulation can be provoked by the injection of urine from pregnant women, the author tried to ascertain whether the ovarian follicles will be ruptured by the transplantation of hypophysis from pregnant rabbits or not. During

16–22 days after copulation 8 females were put under autopsy, 6 of them being found pregnant, 1 in pseudo-pregnancy, and 1 having no corpus luteum.

The hypophysis, removed from each of these 8 animals, was transplanted in the right kidney of other 8 females which were virgin or had never copulated for the last 6 months or a little less. During the period from 7 to 13 days after transplantation these hosts were killed to examine the ovaries. In 7 animals, the implants adhered very well to the tissue of the kidney while in No. 245 the implant slipped out from the initial position.

No. of *Host*	Body-weight *(gr.)*	Number of Large Follicles		Number of Corpora Lutea	
		L.	R.	L.	R.
241	3320	4	7	0	0
242	2980	0	9	0	0
243	2800	3	6	0	0
244	2800	5	7	0	0
245	2700	3	5	0	0
246	2870	0	8	0	0
247	2600	5	1	5	3
248	2500	5	4	0	0

As is shown in this table, ovulation occurred in No. 247, in which the corpora lutea were decidedly fresh.

Discussion.

Oestrous Cycle still exists.

Snyder (1926) states that the vaginal smears from 18 rabbits showed striking cellular changes, especially with regard to the proportion of leucocytes present, but that no regular cyclic recurrence of the smear changes was noted. Kunde and Proud (1929) also describe that as copulation in the rabbit occurs when the cellular content of the vaginal lumen is typical of either of the cyclic changes observed for the rat, the vaginal smear method is ineffective in predicting the oestrous cycle in this animal. The failure in the determination of the oestrous cycle is mainly due to the

following two points:—(i) *There being usually no* corpus luteum in the non-pregnant ovary to regulate the time when the follicles ripen, the vaginal changes are reasonably somewhat irregular and obscure; (ii) the occurrence of the leucocytes has been estimated more conspicuously than that of the nucleated epithelial cells, notwithstanding the fact that the leucocytes are no criterion of oestrum in the rabbit.

In the majority of mammals, although the regulation of the oestrous cycle may be induced by a complex interaction between the corpus luteum and the follicle, the changes occurring in the genital tracts are primarily controlled by the follicle and secondarily by the corpus luteum. ALLEN and DOISY (1924) find that the injection of the follicular contents of the sow's ovary causes the appearence of oestrum in both the spayed and normal but immature rats. PAPANICOLAOU (1924) also testifies with guinea-pigs that the injection of lipoid extract of the follicular and cystic fluids produces action similar to that caused by ALLEN and DOISY, despite the lipoid extract of the corpus luteum tending to bring about *a regression of the large follicles.* ALLEN, DUPER, and HARTMAN (1926) provoke oestrum in the spayed opossum by the injection of the follicular extract of the sow. ASDELL and MARSHALL (1927) find that in some series of experiments with 5 bitches in a state of anoestrum and 1 immatnre and 1 mature spayed rabbit injected with liquor folliculi from the ovary of the pig, the vaginae and uteri of the injected auimals show typical pro-oestrous swelling. In Japan, MIZUNO (1926), SAITO (1927) and others also demonstrate with rats that the principal secretory portion of hormone to provoke oestrum is neither the corpus luteum nor the interstitial tissue, but it is the ovarian follicle itself.

On the other hand the author, as has been stated above, observed by the vaginal smear method oestrum-like changes even in some spayed rabbits for a short time after operation. HALBAN and KOHLER (1914), too, have described that though the existence of the ovary is generally necessary for the normal development and function of the uterus and vagina, and though the removal of the ovaries leads to the disappearance of typical oestrous changes in these organs, the complete spaying does not always prevent the menstrual periodicity for a considerable time afterwards. Still more, STOCKARD and PAPINICOLAOU (1919) state that the *function of the ovary is not* to produce but to control the menstrnation, as oestrum may possibly be due to the existence of a fixed mechanism somewhere outside the ovary.

ALLEN (1921) states that in some mice, where regular oestrous cycles

have been recorded by the vaginal smear method, no fresh corpora lutea are present, while there are many atretic follicles corresponding to the recorded oestrum. HARTMAN (1923) describes with the opossum that if the follicle degenerates the vaginal smear picture becomes obscure and there follows a continuous dioestrous smear, but that if then the follicle reaches maturity the vaginal changes are more or less clear-cut whether the follicle ruptures or not.

It would seem from these explanations that, even if the corpus luteum is absent so long as a series of follicles is developing and apparently active, the mucosa of the uterus and vagina should begin to show signs of hypertrophy and degeneration, and when follicles begin to close the mucosa show normally the dioestrous conditions. For the most part the author concours with BOUIN and ANCEL (1910) who put forward the view that in the rabbit the regulation of the sexual cycle depends upon the interstitial glands, especially upon the fresh atretic follicles, which may be equivalent physiologically to the corpus luteum and have a similar but not so intensely inhibiting effect upon the development of the follicles. There is another theory favourable for the efficiency of the vaginal smear method applied to the rabbit: LOEB (1923) relates that the secretion of the follicles chiefly acts upon the vagina, notwithstauding a comparatively small extent of the influence upon the uterus, while the secretion of the corpus luteum mainly affects the uterus.

From the histological point of view, ASAMI (1920) proves that the atresia in the follicles of the rabbit's ovary occurs in varying sizes and at all stages of the sexual cycle, though he does not fiud any evideuce of typical sexual cycle in this animal. OCHOTERNA and RAMIREZ (1920) state that in the rabbit's ovary the interstitial cells just before oestrum are filled with chondriosomes and at the height of their secretory activity, and that the resulting congestion in the interstitial tissue is helpful for the maturation of the follicles. BELL (1908) mentions that iu the proestrous uterus of the rabbit there occurs the emigration of leucocytes among the epithelial cells of the glands, accompanied by great glandular activity. Moreover, TSU (1924) reports precisely the changes of the vaginal epithelium in the rabbit under several reproductive periods, *i.e.*, before puberty, in the adult, at oestrum, and during pregnancy.

HAMMOND (1925) maintains that in the domestic rabbit no regular oestrous cycle exists, the does always being in a condition of oestrum uuder suitable nutrition at prolonged intervals. If it be so the follicles in the rabbit's ovary must persist in a state of development for a long duration.

But judging from the author's experiments mentioned above as well as from the theories cited here, it becomes clear that in the ovary of the rabbit a series of follicles ripens and degenerates in turn at not so long intervals, and that corresponding to these follicular changes the epithelium of the uterus and vagina also shows signs of the oestrous cycle. The signs of oestrum, therefore, may be reasonably distinguished by the vaginal smear method, although somewhat obscure and variable as compared with the cases of other rodents.

OVULATION DEPENDS UPON HUMORAL CAUSE.

Various theories have up to the present been put forward by some authorities as to the cause of ovulation in the rabbit. WEIL (1873) mentions that ovulation might take place without coition just after the delivery of a litter. IWANOFF (1900) induced pregnancy in the rabbit by artificial insemination soon after parturition. HEAPE (1905) stated that ovulation depends upon the stimulus of coition and it cannot be induced artificially. YAMANE and EGASHIRA (1921) initiate ovulation in 3 of 36 females by artificial insemination without copulation. LUSH (1925) recorded that 4 litters were produced by artificial insemination in the females which had neither delivered a liter nor copulated. In the artificial insemination experiment practised at the oestrum which was presumed by the examination of vaginal cellular contents, the author (1927) succeeded to render 14 out of 37 females pregnant.

HAMMOND (1925), on the other hand, reports that ovulation took place by rubbing the vulva in 4 out of 14 rabbits. HAMMOND and ASDELL (1927) find 3.6 per cent. of cases of ovulation following artificial insemination, which was raised to 13.3 per cent. when the doe was tried to mate with the male though not actually. WALTON and HAMMOND (1928) also maintain that ovulation in the rabbit is provoked by some component of the orgasm which accompanies coition rather than the mechanical stimulus of the penis or the presence of semen in the vagina. It is true that though in the rat and mouse the artificial stimulus made to the cervix often produces pseudo-pregnancy, the stimulus of the penis has no influence on ovulation in the rabbit. Nevertheless, as regards the seminal fluid absorbed through the uterine and vaginal walls the author is of an opinion different from that of WALTON and HAMMOND. The author believes that the semen, injected in a large quantity into the females, has some toxic effects upon the ovary, and when injected in a small quantity, rather accelerates

the growth of the follicles. MATSUYAMA (1919) maintains that in the parabiosis of the male and female rats the initial changes occurred in the sexual glands, especially in the ovary, the follicles of which were strikingly developed and succeeded by the atresia, while these phenomena were not observed in the parabiosis of two females. DITTLER (1920) says that the rabbit became infertile by the intravenous injection of the rabbit's semen, and that by this spermatoxin the ripening and rupture of the follicles were not inhibited. FUKUYAMA (1923) reports that in the female rat the growth of follicles of all sizes was at first accelerated by the testis-transplantation and then they underwent degeneration. POMMERENKE (1928) mentions that, through the injection of seminal fluid into the female rabbits, antibodies were produced which caused the serum and vaginal secretions of the females to be toxic to the spermatozoa of the rabbit and rat, whereas such injection had no effect on the oestrous cycle. MORIMUNE (1927) describes that when human sperm was injected into the female rat the development of follicles even in the deep portion of the ovary was markedly quickened, and they degenerated soon afterwards. The author's experiments of the injection or transplantation of testicular and prostatic substances also proved that the follicles of all sizes develop rapidly by the stimulus of these male sexual hormones. This fact suggests that the seminal fluid absorbed through the genital tracts exerts some influences on the follicular system.

HIROSE (1921) and ADACHI (1623) already induced ovulation in the rabbit by injecting the emulsion of the hnman placenta or aciniform blood-moles. In the light of recent researches with female sexual hormones a great advancement has been made in the study of the cause of ovulation in the rodent. SMITH and ENGLE (1927, 1929) ZONDECK and ASCHHEIM (1927, 1028), ENGLE and MERMOD (1928), LIPSCHÜTZ and PAEZ (1928), LIPSCHÜTZ, KALLAS and PAEZ (1929), EVANS and SIMPSON (1929), MARTINS (1929), YAMASAKI (1929) and some others in Japan, COURRIER, KEHL and RAYNAND (1929), KALLAS (1929), STRICKER and GRIETER (1930), etc., all contributed towards the progress of investigation of the female sexual hormones. They produced super-ovulation in the rats and mice by the transplantation of mammalian hypophysis or by the injection of urine from pregnant women. FRIEDMAN (1929) says that it was impossible to provoke ovulation in the rabbit by the transplantation or by the intraperitoneal injection of fresh rat hypophysis. The author's experiment, however, shows that ovulation occurs by the transplantation of hypophysis from the pregnant rabbit.

Regarding the anterior lobe of the pituitary body there are many

important problems left untouched, *i.e.*, the relation between the hormone from fresh hypophysis and that from pregnant woman's urine, between the hypophyseal extract and the ovarian extract, and the difference in the properties of this hormone in the pregnant and the non-pregnant animal. Apart from these complex problems, it is at least reasonably acknowledged that the cause of ovulation in the rabbit depends upon the humoral mechanism but not upon the transmission of the nervous system. FRIEDMAN'S recent work (1929) is fortuitously coincident in part with the author's experiments, *viz.*, FRIEDMAN, finding that the removal of the whole genital tracts from the female rabbit does not prevent ovulation and that ovulation may occur in the transplanted ovarian tissue, also maintains that following copulation there occur in the humours of the doe some changes capable of provoking ovulation.

Summary.

(1) In the rabbit the oestrous cycle, though rather obscure as compared with that of other rodents, can be determined to a certain extent by the examination of the vaginal smear.

(2) Among three different cells which make their appearance in the vaginal smear only the nucleated epithelial cells may be used as criterion for the determination of the oestrous stage.

(3) The appearance of the nucleated epithelial cells in the vaginal lumen is in close relation to the development of ovarian follicles.

(4) Judging chiefly from the frequency of the nucleated epithelial cells in the vaginal smear, the duration of the oestrous cycle extends from 3 to 12 days and is about 7 days on an average.

(5) The cornified epithelial cells are rarely found in abundance in the vaginal smear. This fact can also be proved by the examination of the serial sections of the genital tracts.

(6) Not only do the leucocytes appear during the normal oestrous period, but they are observable in large numbers at irregular intervals during the time of pregnancy and lactation or even before puberty.

(7) It is also possible to distinguish the difference between complete and incomplete ovariotomy by the vaginal smear method.

(8) Oestrum-like phenomena sometimes take place at the time of pregnancy and lactation, or even after spaying in some animals. However, ovulation never occurs spontaneously at any reproductive period in any animal.

(9) Ovulation takes place following copulation in the rabbit comple hysterectomized or vagina-anaesthetized.

(10) Ovulation, though at a low percentage, can be induced in normal doe either by means of the artificial insemmation or by the tra plantation of fresh hypophysis from a pregnant rabbit.

(11) Testicular substance, when injected or transplanted, acceler the growth of the ovarian follicles to a certain extent.

(12) The cause of ovulation appears to depend upon some hum mechanism.

Addendum.

Most recently (July, 1931) the author provoked ovulation in 6 of 29 rabbits by the injection of the hypophyseal emulsion obtained fr the *pregnant* and *non-pregnant* rabbits.

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EXPLANATION OF PLATES.

PLATE XVIII.

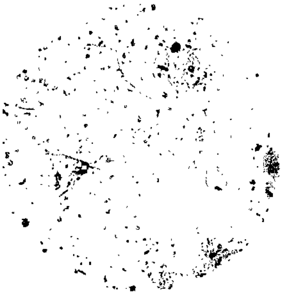
1. Cornified epithelial cells in the vaginal smear obtained from a normal rabbit. These cells are rarely observed in abundance at any stage of reproductive periods.
2. Nucleated epithelial cells in the vaginal smear obtained from a normal rabbit. The occurrence of these cells corresponds to the development of ovarian follicles.
3. Polymorphonuclear leucocytes in the vaginal smear obtained from a normal rabbit. A number of leucocytes appears at irregular intervals in the rabbit.
4. Leucocytes and spermatozoa in the vaginal smear obtained from a rabbit 4 hours after copulation.
5. Transverse section of uterus of a normal rabbit at oestrous stage just before copulation. Only scattered leucocytes are observed in the lumen, no cornified epithelium being present.

PLATE XIX.

6. Longitudinal section of the remaining left-side ovary found in No. 116, to show blood-follicles and atretic follicles but no healthy follicle and germinal epithelium.
7. Longitudinal section of the remaining right-side ovary found in No. 172, to show corpora lutea and atretic follicles but no germinal epithelium.
8. View of a liver with the transplanted testis.
9. Transverse section of a normal young testis, 3-months-old.
10. A part of the transplanted testis, to show markedly degenerated seminal tubules.
11. The same, to show completely degenerated seminal tubules.

PLATE XX.

12. Transverse section of the left-side ovary in No. 119, to show a slightly increased number of follicles by the first effect of testis-transplantation.
13. Longitudinal section of the right-side ovary in No. 119, to show the same effect.
14. Longitudinal section of the right-side ovary in No. 122, to show a greatly decreased number of follicles by the second effect of testis-transplantation.
15. View of 2 kidneys with the transplanted hypophysis.
16. Ovaries of No. 247, to show newly formed corpora lutea by the effect of transplanted hypophysis.



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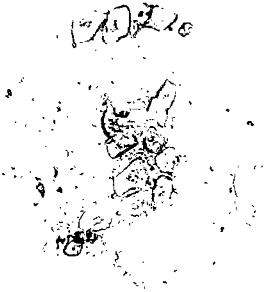

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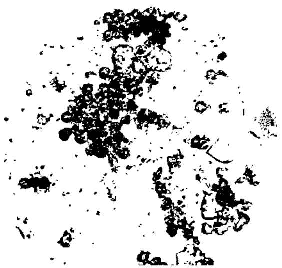

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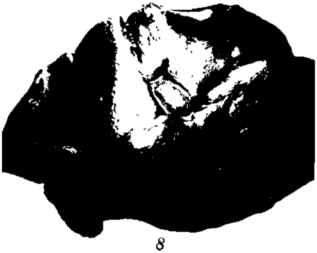
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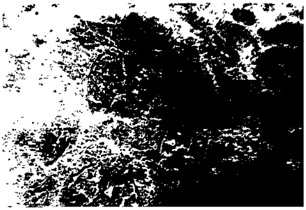

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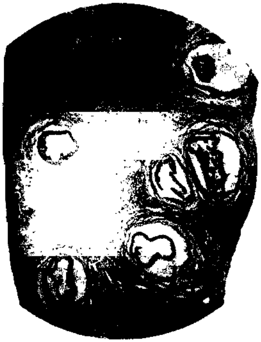

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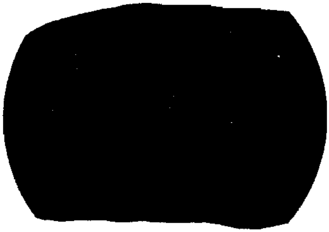

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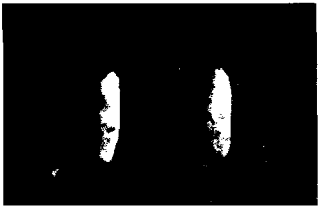

On the Conjugation of the Gametes and the Development of the Zoospores in Ulvaceae.

By

Kiichi Miyake and Hiroshi Kunieda.

With Plates XXI–XXIV.

Introduction.

In his well known researches on the zoospores of algae THURET (1850) made some observations on the zoospores and gametes of Ulvaceae. He reported that there are two forms of the "zoospores," the one having four cilia, the other two, and that the two-ciliated "zoospores" of *Enteromorpha clathrata* conjugate in pairs.

ARESCHOUG (1875) made some more careful observations on the conjugation of gametes in *Enteromorpha compressa* and found that on a certain day almost all gametes conjugated, but on another day almost none. In explanation of this remarkable fact he presumes that the gametes are likely to be male and female.

The conjugations of gametes were also observed by DODEL-PORT (1877) in *Enteromorpha clathrata* and by STRASBURGER (1878) in *Enteromorpha compressa*.

REINKE (1878) studied the conjugation of the gametes and development of the zygotes in *Monostroma bullosum*. Many years later CHODAT (1894) made some observations on the development of the zygotes of the same plant and obtained somewhat different results from those of REINKE.

In 1907 SCHILLER published the results of his studies on the development of *Ulva Lactuca*. He reports that there are three kinds of gametes in *Ulva*, the largest macrogametes do not conjugate being incapable of developing, and the medium-sized parthenogametes can develop parthenogenetically, while the smallest microgametes conjugate and develop to a new plant.

More minute studies on the cytology and biology of the Ulvaceae have been made by CARTER (1926). She states that in *Monostroma latissi-*

mum the plants are dioecious, gametes from any single plant of this species failing to conjugate with each other, and producing zygotes only when they are brought into contact with gametes of a plant of the opposite sex.

HARTMANN (1929b) reported that the sharply separated sexuality was proved in *Enteromorpha compressa* by HÄMMERLING and in *Enteromorpha ramulosa* by himself. He states that in *Enteromorpha* as in *Ulva* there are large 4-ciliated zoospores and small 2-ciliated gametes which are formed on different, but externally not distinct plants.

FÖYN (1929), working on *Ulva Lactuca*, has found, contrary to the results obtained by SCHILLER (1907), that there is a strictly differentiated sexuality, as has been proved by HÄMMERLING (HARTMANN 1929a). He was not able to distinguish the three kinds of gametes as mentioned by SCHILLER.

In the early spring of 1930 we started a study in the Marine Biological Station of the Tokyo Imperial University at Misaki with the object of acquiring some knowledge of the sexuality and cytology of the Ulvaceae. Although the study has not yet been completed, we have made some interesting observations on the conjugation of the gametes and the development of the zoospores in *Monostroma*, *Enteromorpha* and *Ulva*, which we are now going to report in the present paper.

Monostroma.

A certain *Monostroma* species which grows abundantly from winter to early summer in the vicinity of Misaki was used for our observations. In the early part of May we collected some *Monostroma* plants color of which had been turned more or less to yellowish-green. We rinsed them and placed them in sea-water, each plant in a separate dish. On the next morning we found that the gametes were liberated from most of the plants. SCHILLER (1907) in *Ulva* and CARTER (1926) in *Monostroma* reported that the gametes are liberated and swarm in the early morning. We found that the time of the liberation of the gametes is generally between 4.30 and 7 a.m., but sometimes it takes place later in the morning lasting till noon. The liberation of the gametes continued until late in June, the best conditions being found in about the first 10 days of the same month. From the beginning of May to the end of June we could not come across even a single zoospore.

The structure of the gametes has been described in *Ulva* by SCHILLER (1907) and in *Monostroma* by CARTER (1926). On the whole we can con-

firm their observations. While the gamete is actively moving, it is elongated pear-shaped or more or less fusiform, having two long cilia attached to the anterior end. It is usually pointed at the anterior end and more or less rounded at the posterior end (Plate XXI, Figs. 1–3). We have, however, often found the gametes with both ends pointed, so that they give a fusiform appearance, as has been reported by SCHILLER in *Ulva* (Figs. 4–7). Among the latter we have come across not infrequently a gamete with a short cilium-like appendage at the pointed posterior end (Figs. 6, 7). The anterior part of the gamete is colorless, while the posterior half is green, being occupied by a large chloroplast with reddish eye-spot. Many granules are seen scattered all over the body of the gamete except in the anterior end where very few of them are found. Under high magnification in which all of our sketches of the gametes were made, the outline of the chloroplast is not very distinct, so only the conspicuous granules and eye-spot are shown, and any indication to show the presence of the former is omitted in the figures.

The variation in size of the gametes has already been noticed by ARESCHOUG (1876) in *Enteromorpha*. SCHILLER (1907) has distinguished three kinds of gametes with corresponding difference of size in *Ulva*. He, however, does not seem to have found any difference in size in connection with their sexuality. CARTER (1926), on the other hand, found some difference of size according to sex in *Monostroma*. She reports; "a very slight difference in size of the gametes was discerned, the smaller gametes being presumably ♂, and the larger ones ♀. There is considerable variation in size of the ♂ and ♀ gametes, so that some of the larger ♂ gametes may be so large as the average ♀ gametes or even larger; whilst some of the smaller ♀ gametes may be equal to or even smaller than the average ♂ gametes in size. On the whole, however, the ♀ gametes are slightly larger than the ♂ gametes." FÖYN (1929) found the variation in size of the gametes in *Ulva*, although he failed to distinguish three kind of gametes as mentioned by SCHILLER. He, however, was not able to identify the sexual difference in size of the gametes. Although there was a considerable variation in size of the gametes, we failed to find any difference in size in connection with their sexuality.

Beside the usual forward movement the gametes were often seen to rotate clockwise facing the anterior part on the slide-glass. A similar rotating movement of gametes has been described in *Ulva* by SCHILLER. As the motion becomes less active, they become more or less oval and finally round themselves up when the motion ceases as in Figs. 8–11.

As soon as the gametes are liberated from the frond, they collect around the lightest part of the vessel, forming a green streak or cloud near the surface of the water, as mentioned by CARTER (1926). We made some experiments on the sensitiveness of the *Monostroma* gametes to light. When the gametes are transferred into the watch-glass which is placed in front of a window, they will be seen to seek at once the lightest part of the vessel forming a green streak or cloud. If the lightest part is smeared with Indian ink, the streak at once changes its position, seeking other lighter parts. Now, if the watch-glass is turned round 180° so that the streak is made to occupy the darkest part of the vessel, which is the farthest side from the window, at once the gametes begin to recede and the green cloud is seen moving toward the light. In about 2 minutes the gametes again take possession of the lightest region, forming the same streak as before. By changing the position of the watch-glass this movement forward and backward continues for a long time, sometimes for 4 hours, although the motion of the gametes becomes slower as the time of rotating the watch-glass increases.

Early in the morning on June 8th, 1930 we found that the gametes were abundantly liberated from 4 out of 51 plants which we had collected on the previous day, each plant being put in a separate dish. On the 9th, 4 more plants gave gametes, on the 19th 16 plants were added, and on the 11th 26 more plants, that is to say, all the plants gave gametes abundantly except only one. During these days, however, we did not observe any zoospore at all. So many gametes escaped from all these plants that we could very easily conduct experiments on their reactions to light and sexuality.

CARTER states that the *Monostroma* plant is dioecious and produces gametes of one sex only, which are incapable of conjugating between themselves. We also found that only the gametes of the different sexes can unite, while no conjugation takes place among the gametes of the same sex. When two gametes of different sexes meet, they unite with each other at their anterior ends, lying side by side as shown in Figs. 12–16 and a case in which the fusing gametes meet end to end is rarely met with.

In *Ectocarpus* several male gametes are attracted to a single female gamete forming a conjugating group or so-called "Gruppenbildung." A similar "Gruppenbildung" was observed by HARTMANN (1929b) in *Chaetomorpha* and by FÖYN (1929) in *Cladophora*. HARTMANN, however, failed to find the "Gruppenbildung" in the conjugating gametes of *Enteromorpha compressa*, while he observed it in *Enteromorpha ramulosa*. We were not

able to observe any case of the "Gruppenbildung" in *Monostroma*.

The zygote, which has four cilia, continues to move about actively, but more slowly than the gamete, and its body oscillates so peculiarly that it is easily distinguished from the gamete with the low power of the microscope. The fusion begins to take place at the anterior end, and then gradually throughout the whole body. At first the two reddish eye-spots are on the outer lateral sides of the zygote, but later they move toward the bottom as shown in Fig. 17. The zygote continues to move for about 3 or 4 hours without losing its cilia, while the gametes swarm very actively for 3–12 hours, without losing the conjugating capacity. During this time the shape of the zygote gradually changes, and the pear-shaped zygote becomes finally spherical (Figs. 18–20). The zygote then loses its cilia and comes to rest (Figs. 21, 22). Soon it begins to aquire a cell-wall, retaining the two eye-spots for a few days (Fig. 23).

It is possible to determine from macroscopic observation whether the gametes are of the same or of the different sex. Whenever the gametes from two plants of the same sex are put together in turn in a watch-glass, all of them seek the lightest part, forming a streak. On the contrary if they are of the different sexes, within two or three minutes we can see two streaks which are very much like each other, both at the lightest and the darkest parts of the vessel. Microscopic examination shows that the streak in the lightest part consists entirely of the non-conjugated gametes, and the other is entirely of the conjugated ones.

When a streak is formed by the gametes of one sex along the lightest side of a watch-glass, if we bring those of the opposite sex into the other side of the vessel, then the latter, swarming at once to the lightest part, gathers in a cloud just behind the former and soon the both gametes conjugate forming a thick cloud which consists of the zygotes. The thick cloud begins to recede, and soon seeks the darkest part of the vessel, forming a streak, while the non-conjugated gametes remain at the lightest part.

If the watch-glass is now carefully turned round 180°, each streak at once begins to recede, and again takes possession of the darkest and lightest parts respectively as before. We found that the return of the gametes to the lightest region of the watch-glass is attained within 2 or 3 minutes which is about three times as fast as the zygotes recede to the darkest part. This movement caused by rotating the watch-glass can be repeated even after 2 hours of mixing the gametes of the opposite sexes.

Table I shows the results of all possible combinations of the gametes

of the 5 plants we obtained on June 11th in which we observed the gametes under the microscope. There was no morphological difference between the gametes of the different sexes. We may denote here the plants No. 11 and 14 belonging to one sex with + and the plants No. 2, 12 and 16 which belong to the other sex with −.

TABLE I.

Results of combinations of 5 plants in *Monostroma*.

	11	14	2	12	16
11	−	−	+	+	+
14	−	−	+	+	+
2	+	+	−	−	−
12	+	+	−	−	−
16	+	+	−	−	−

In Table II, the results of all possible combinations of 18 plants are given. The plants No. 4, 12, 13, 14, 17, 19, 36, 40 belong to one sex (+), and the plants No. 1, 3, 6, 15, 16, 37, 38, 43, 46, 49 to the other sex (−).

We repeated the experiments several times and the strict besexuality of *Monostroma* plants was conclusively proved. On June 19th, we found that out of 19 plants 9 belonged to the one sex and 10 to other; on June 22nd 13 plants belonged to the one sex and 12 to the other out of 25 plants; and on June 29th, 3 belonged to the one sex and 3 to the other out of 6 plants.

When the equal amount of the gametes of both sexes are mixed togather, there always remains some amount of non-conjugated gametes. They are probably the gametes of weaker sexual affinity of both sexes, and can conjugate only with the gametes of stronger sexuality.

Table II.

Results of combinations of 18 plants in *Monostroma*.

	4	12	13	14	17	19	36	40	1	3	6	15	16	37	38	43	46	49
4	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
12	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
13	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
14	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
17	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
19	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
36	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
40	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
3	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
15	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
16	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
37	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
38	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
43	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
46	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
49	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

As to the germination of the zygote there is much diversity in the observations of the previous investigators.

REINKE (1878) observed that in *Monostroma bullosum* the zygote, after acquiring a cell-wall, gradually increases in size for 7 or 8 weeks, after which germination takes place in some individuals, but in others germination did not take place even after 5 months. In the germination of the large spherical zygote the contents first divide into eight cells, arranged round the periphery. Cell-division proceeds in such a way as to preserve the structure of a hollow sphere, which represents the young *Monostroma* plant.

CHODAT (1894), working on the same plant, found that the zygote germinates immediately and not after a period of rest as described by REINKE. He states that the germinating zygote first forms a simple filament of four cells, which by further cell-divisions gives rise to a new *Monostroma* plant.

In *Monostroma latissimum* CARTER (1926) failed to observe the germination of the zygote. She states that the zygote increases tremendously in size for about forty days, and forms a large thick-walled resting cyst about 50 μ in diameter, which does not germinate for several months.

As has been mentioned before, the conjugated gametes or zygotes accumulate at the darkest part of the vessel, forming a green streak or cloud. Using a sterile pipette a part of the cloud was transfered to a sterilized dish containing the clean sea-water. After several hours the zygotes gradually settle down in the bottom of the vessel. The zygotes were examined from time to time.

The restiug zygote is soon invested with a cell-wall. The two eye-spots are distinctly visible, although they disappear in a few days (Figs. 23–24). Sometimes the eye-spots persist much longer, and the cases are not rare in which at least one of them may be seen even after two weeks. Fig. 25 shows a zygote with one eye-spot as observed after 18 days.

With the increase in size of the zygote its wall gradually thickens and the contents become dark green (Fig. 26). For 4–5 weeks the zygote shows no sign of germination, being now a spherical body with a thick cell-wall about 15–20 μ in diameter (Fig. 27).

After about 40 days a few zygotes showed a sign of germination by dividing into two (Fig. 28). It further divides again and forms a three-celled sporeling (Fig. 29). In the 60-days old cultures we have found a few 2- or 3-celled sporelings among the great majority of ungerminated zygotes. After that no further changes were observed.

Enteromorpha.

While we were collecting the marine algae at a little island called Jyogashima near the town of Misaki in the middle of July 1930, we happened to meet with many fertile fronds of *Enteromorpha*. This undetermined species of *Enteromorpha* was used for our observations. The alga is slender and filamentous measuring about 7 to 20 cm. in length. The fertile part of the frond is easily distinguishable by its orange color.

If the fronds are collected and kept in a moist dish which is covered over so as to prevent them from drying up, the motile gametes and zoospores can be released at any time for 2 or 3 days, by putting them into sea-water. In dealing with the gametes, we employed the same method as described in *Monostroma* with good results. The fronds of *Enteromorpha*, however, are very slender as compared with those of *Monostroma* and liberate the motile cells in a small amount. We have, therefore, made all the experiments under the microscope, and not in a large scale as tried in *Monostroma*.

The gametes of *Enteromorpha* are similar to those of *Monostroma* in form and structure, and they show a considerable variation in size (Plate XXII, Figs. 30–34). No difference in size was found in connection with their sexuality.

Using a fine pipette, we put on the slide-glass a green drop of the gametes which form at once a small streak at the lightest edge of the drop. When a drop of the gametes of the different sex is placed very carefully at the opposite edge of the drop, the conjugation at once takes place and soon the zygotes are found accumulating at the darkest edge of the drop, forming a small streak (Figs. 35–39). The sensitiveness of the gamete to light is also remarkable, but it seems to be somewhat weaker as compared with that of *Monostroma*.

Some gametes of *Enteromorpha* conjugate side by side just as is generally the case in *Monostroma*. There are, however, others which unite at their anterior ends opposite to each other (Figs. 35). In the latter case two conjugating gametes soon assume a cocoon-like shape and later become a round body (Fig. 40). The "Gruppenbildung" of the gametes which was reported in *Enteromorpha ramulosa* by HARTMANN (1929b) was never met with in this plant. The zygote soon loses its cilia and comes to rest (Figs. 41–43). We did not follow the germination and development of the zygote any further.

On July 11th and 15th 1930, we carried on our experiments to see

TABLE III.

Combinations of 5 plants in *Enteromorpha* observed on July 11th.

	1	2	5	12	4
1	−	−	−	−	+
2	−	−	−	−	+
5	−	−	−	−	+
12	−	−	−	−	+
4	+	+	+	+	−

TABLE IV.

Combinations of 6 plants in *Enteromorpha* observed on July 15th.

	7	17	12	13	27	28
7	−	−	+	+	+	+
17	−	−	+	+	+	+
12	+	+	−	−	−	−
13	+	+	−	−	−	−
27	+	+	−	−	−	−
28	+	+	−	−	−	−

whether or not the sharp separated sexuality exists in our *Enteromorpha* just as in *Enteromorpha compressa* and *Enteromorpha ramulosa* reported by HARTMANN.

Table III gives the results of all possible combinations of the 5 plants. Of these, four plants No. 1, 2, 5, 12 belong to + group, and only one plant No. 4 to – group.

Table IV shows all possible combinations of the 6 plants. Two plants No. 7, 17, belong to + group, and four plants No. 12, 13, 27, 28 to – group.

Although HARTMANN (1929b) observed in *Enteromorpha ramulosa* a case of relative sexuality (HARTMANN 1925), we have never met with such a case in our plants. The individuals and gametes are therefore strictly differentiated into + and – sexes in our *Enteromorpha* as in *Monostroma.*

In these experiments we had a chance to find the large 4-ciliated zoospores. The zoospores are very different from the gametes in size, structure and behavior as shown in Figs. 44–46. They are much larger than the gametes and are formed on the different plants which we can not tell externally from the gamete-bearing individuals. Escaping through a pore in the parent cell, more slowly in motion compared with the gametes, they swarm about at the lightest part of the watch-glass and form a streak like the gametes.

The streak formed by the zoospores seems to be somewhat different from that of the gametes. The outline of the former is somewhat obscure at the inner margin, while that of the latter is very clear and distinct. The reason why the streak of the zoospores makes such an obscure appearance is due to its weaker positive phototax compared with that of the gametes.

After swimming for some time the zoospore comes to rest, losing its cilia (*Fig.* 47). Now it becomes round, and acquires a cell-wall which gradually thickens. On the following day the eye-spots are still distinctly visible in some of the zoospores, while in others they seem to have disappeared (Fig. 48). Soon the pyrenoid becomes clearly visible (Fig. 49). Within 2 or 3 days the round zoospore elongates and divides into two (Figs. 50–52). The further cell-division is followed by the elongation of the filament. After 5 or 6 days we can see that the thread consists of 3 or 4 cells each containing a pyrenoid (Figs. 53–55). The further development has not been followed.

Ulva.

Two species of *Ulva* grow around the Misaki Biological Station. One of them which we have used for the present observation is very much like

Ulva Lactuca so far as its external characters are concerned, but not identical, and we are obliged to leave it undetermined at present.

From the latter part of June till the end of July 1930, we examined several hundred plants of *Ulva*. Of these only 8 plants gave gametes, and the others were mostly zoospore-bearing individuals. The liberation of the zoospores took place about the latter part of July. No external distinction was observed between the two kinds of plants as mentioned by FÖYN (1929) in *Ulva Lactuca*.

The gametes are liberated in the early morning as stated by SCHILLER (1907) and FÖYN (1929), and always gather at the window-side of the dish, forming a streak of yellowish-orange color.

Their structure and their sensitiveness to light are about the same as those of *Monostroma* and *Enteromorpha*. As in *Monostroma* and *Enteromorpha* the gamete of *Ulva* is elongated pear-shaped or almost fusiform. It is usually pointed at the anterior end and more or less blunt at the posterior end (Plate XXIII, Figs. 56–58). There were often found gametes with both ends pointed as mentioned by SCHILLER (Figs. 59, 60), and among them we have come across not rarely the gamete with a cilium-like appendage at the posterior end (Fig. 60). The gametes vary considerably in size, although the three kinds of gametes as mentioned by SCHILLER were not distinguishable. As the motion becomes less active, the gamete becomes more or less oval as shown in Fig. 61.

In the early morning of July 3rd 1930, the gametes were released in large numbers from 6 plants and attempts were made to make all possible combinations of them by mixing two at a time to study their sexual behavior as described in *Monostroma*. None of the gametes, however, was found to conjugate, as they were all of the same sex.

We have paid more close attention to the zoospore-bearing plants. The fertile frond can easily be distinguished by its yellowish-green color. The change of color first begins at the margin of the frond and extends gradually towards the base. The number of the zoospores formed in each cell seems to vary from 4 to 16, the more common number being from 8 to 16. The ripe zoospores are seen moving actively within the parent cell, and sooner or later escape from a pore in the center of the cell-wall.

After they have escaped, the zoospores gradually sink and scatter about at the bottom of the vessel. They do not show any distinct reaction towards light, although they seem to have a slight negative phototactic property. The zoospores is much larger than the gamete, and contrary to our expectation, it has two cilia instead of fonr unlike that of *Ulva Lactuca*

reported by SCHILLER and FÖYN (Figs. 62–67).

The zoospore soon loses its cilia and gives rise to a spherical body, which gradually increases in size with the thickening of its wall (Figs. 68–73). The eye-spot is still visible for a few days (Figs. 70, 71). In two or three days it begins to germinate by pushing out a protuberance (Figs. 74, 75). Now the cell divides transversely into two (Figs. 76, 77), one of which elongates and forms the primary rhizoid (Fig. 78). By further cell-division and elongation the young sporeling develops usually into a simple filament, consisting of a single row of cells with a rather long rhizoid cell (Figs. 79–85).

We have often found the young sporeling consisting of a single row of cells of nearly equal size and without the primary rhizoid (Plate XXIV, Figs. 88–92). Such sporeling seems later to bend at the middle of the filament and from there to give out a branch or rhizoid (Figs. 93–96). Although most of the branched filaments have a rhizoid, we have sometimes met with the branched sporelings without any rhizoid (Figs. 97, 98).

The primary rhizoid is almost colorless, whilst the other cells of the filament are deep green. The former is sometimes branched and often divided into 2 cells (Figs. 84, 87, 96).

When the number of cells in a filament increases to 10 or more after successive transverse divisions, the longitudinal divisions begin to take place. As reported by SCHILLER soon the primary rhizoid seems to degenerate and the secondary rhizoids are formed. The most advanced young sporelings we have observed are shown in Figs. 99–101. The further development of the sporelings were not observed.

Summary.

1. In *Monostroma* the plants are dioecious, having a strictly differentiated sexuality. Only the gametes from plants of different sexes conjugate amongst themselves.

2. There is a considerable variation in size and form of the gametes, but any difference according to the sex was not observed.

3. The gamete is elongated pear-shaped or more or less fusiform, having two long cilia attached to the anterior end. It is usually pointed at the anterior end and more or less rounded at the posterior end. Gametes with both ends pointed were, however, often found.

4. Both the gamete and zygote are sensitive to light, the former being positively phototactic, while the latter shows a negative phototactic reaction.

5. The zygote is invested with a thick cell-wall and gradually increases in size. It does not germinate for many days. After about 40 days a few zygotes showed signs of germination and formed 2- or 3-celled sporelings.

6. In *Enteromorpha* there is a strictly differentiated sexuality, and the conjugation takes place only between the gametes of different sexes.

7. As in *Monostroma*, the gamete of *Enteromorpha* is positively phototactic, while the zygote shows a negative phototactic response.

8. The form and structure of the gametes of *Enteromorpha* are similar to those of *Monostroma*. Although there is a considerable variation in size and form of the gametes, no difference in connection with their sexuality was observed.

9. The zoospore is 4-ciliated and much larger than the gamete. It shows a positively phototactic response, although it is slightly less sensitive to light compared with the gamete.

10. The resting zoospore germinates within 2 or 3 days, and after 5–6 days 3- or 4-celled sporelings were obtained. The further development was not followed.

11. In *Ulva*, the form and behavior of the gametes are similar to those of *Monostroma* and *Enteromorpha*.

12. The zoospore is much larger than the gamete and has two cilia instead of four as previously reported.

13. The resting zoospore with a thick cell-wall begins to germinate in 2 or 3 days, by pushing out a protuberance. The cell divides transversely into two, one of which elongates and forms the primary rhizoid.

14. By further cell-divisions and elongation the young sporeling develops into a simple filament consisting of a single row of cells with a rather long rhizoid.

15. When the number of cells in a filament increases to ten or more after successive transverse divisions, the longitudinal divisions begin to take place.

16. The primary rhizoid soon degenerates, and the secondary rhizoids are formed.

17. The young sporelings were often found consisting of a single row of cells of nearly equal size and without a primary rhizoid. Such sporelings seem to develop later into those sporelings consisting of branched filaments with or without primary rhizoids.

ADDENDUM.

Since this paper has gone to the press a paper by H. KYLIN—Über Heterogamie bei *Enteromorpha intestinalis*. (Ber. d. Deut. Bot. Ges., Bd. 48, 1930) has appeared. KYLIN has observed the heterogamic gametes in *Enteromorpha intestinalis*. According to him the female gamete is about 6.0–6.8 μ in length and 3.0–3.5 μ in breadth, while the male gamete is about 5.0–5.6 μ in length and 1.8–2.2 μ in breadth.

In the early part of April 1931 we have observed the same sexual difference in the gametes of *Ulva* sp. Whenever the two kinds of gametes coujngate, one of them were always larger than the other. The gamete of *Ulva* which we describe and figure in the present paper is probably that of the male. In other species of *Ulva*, however, the gametes of different sex were of the same size.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida and are reduced to $\frac{2}{3}$ the original size in reproduction.

PLATE XXI.

Monostroma.

Figs. 1–5. Various forms of gametes. ×2000.

Figs. 6, 7. Gametes with a cilium-like appendage at the posterior end. ×2000.

Figs. 8–11. Resting gametes; they become rounded up when the motion ceases and finally lose the cilia as in Fig. 11. ×2000.

Figs. 12–18. Various stages in the conjugation of gametes. ×2000.

Figs. 19, 20. Young zygotes with four cilia. ×2000.

Figs. 21, 22. Resting zygotes. ×2000.

Fig. 23. Zygote on the second day. ×2000.

Fig. 24. The same on the fourth day. ×2000.

Figs. 25, 26. 18-days old zygotes, in one of which an eye-spot is still visible. ×2000

Fig. 27. About 40-days old zygote. ×1200.

Figs. 28, 29. Young sporelings 43 days old. ×1200.

PLATE XXII.

Enteromorpha.

Figs. 30–33. Various forms of gametes. ×2000.

Fig. 34. Gamete with a cilium-like appendage at the posterior end. ×2000.

Figs. 35–38. Various stages in the conjugation of gametes. ×2000.

Fig. 39. Conjugating gametes, cilia not being visible. ×2000.

Fig. 40. a-d. Succesive stages of the conjugation of gametes as observed under the microscope. ×2000.

Fig. 41. Young zygote with four cilia. ×2000.

Figs. 42, 43. Resting zygotes. ×2000.

Figs. 44–45. Zoospores. ×2000.

Fig. 46. Zoospore just before it comes to rest. ×2000.

Fig. 47. Resting zoospore. ×2000.

Fig. 48. Resting zoospore on the 2nd day. ×2000.

Fig. 49. The same with the pyrenoid. ×2000.

Figs. 50, 51. Germination of zoospores. ×2000.

Fig. 52. 2-celled sporeling. ×1200.

Figs. 53, 54. 3-celled sporelings. ×1200.

Fig. 55. 4-celled sporeling. ×1200.

Plate XXIII.

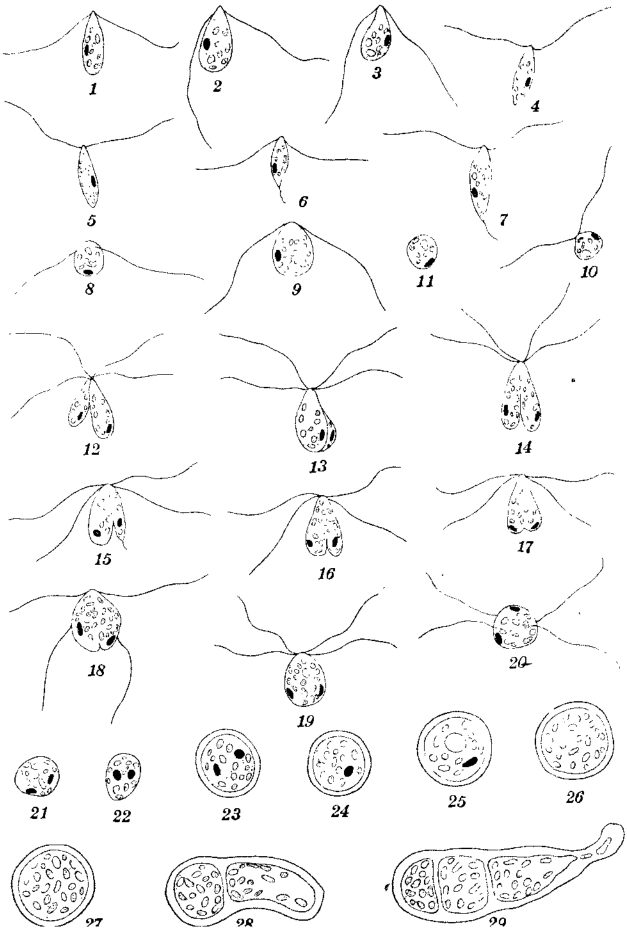
Ulva.

Figs. 56–59. Various forms of gametes. ×2000.
Fig. 60. Gamete with a cilium-like appendage at the posterior end. ×2000.
Fig. 61. Resting gamete. ×2000.
Figs. 62–65. Various forms of 2-ciliated zoospores. ×2000.
Figs. 66, 67. Zoospores just before they come to rest. ×2000.
Figs. 68, 69. Resting zoospores. ×2000.
Figs. 70, 71. Resting zoospores on the 2nd day. ×2000.
Fig. 72. Resting zoospores, a slightly later stage. ×2000.
Fig. 73. Resting zoospore, much enlarged. ×1200.
Figs. 74, 75. Germination of zoospores. ×1200.
Figs. 76, 77. 2-celled sporelings. ×1200.
Figs. 78, 79. 3-celled sporelings. ×600.
Fig. 80. 4-celled sporeling. ×600.
Figs. 81, 82. 5-celled sporelings. ×600.
Figs. 83–85. More advanced stages of the sporelings. ×600.

Plate XXIV.

Ulva.

Figs. 86, 87. Young sporelings in about the same stages as above. ×600.
Figs. 88–92. Successive stages in the development of young sporelings without primary rhizoid. ×600.
Fig. 93. The same more advanced, with a bending at the middle of filament. ×600.
Figs. 94–96. The same still more advanced, giving out a branch or rhizoid. ×600.
Fig. 97. Branched sporeling without any rhizoid. ×600.
Fig. 98. The same, slightly older stage. ×280.
Figs. 99–101. The more advanced stages of young sporelings. ×280.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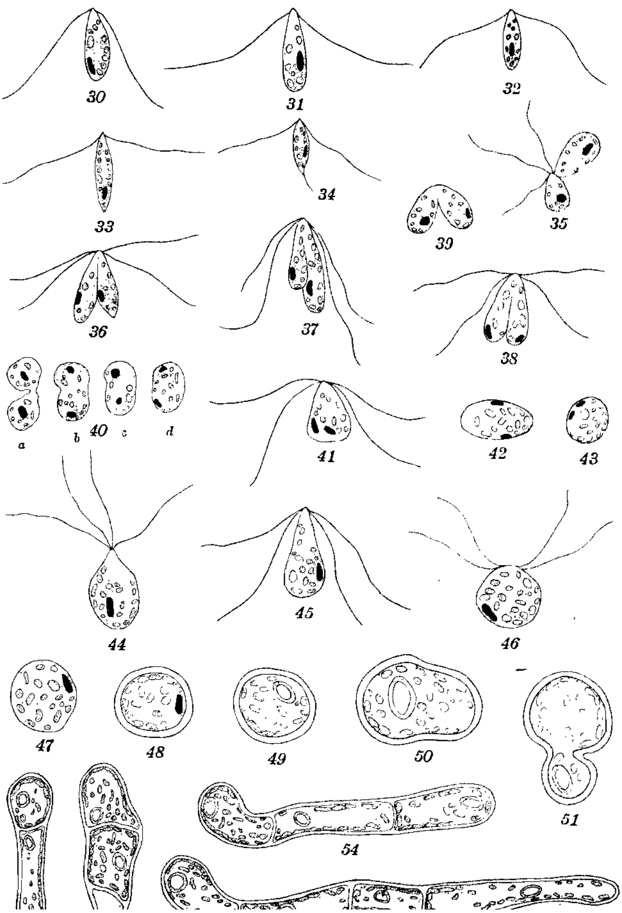

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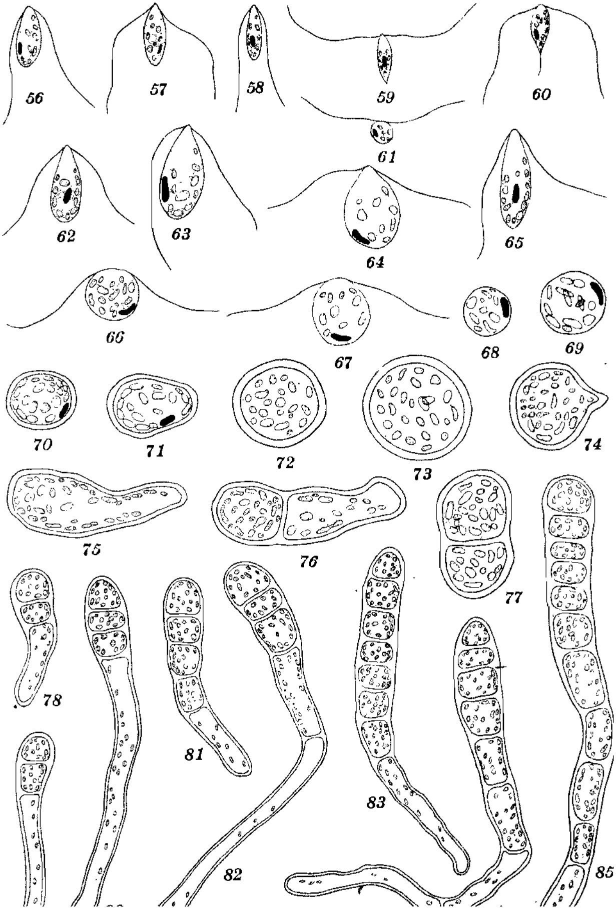
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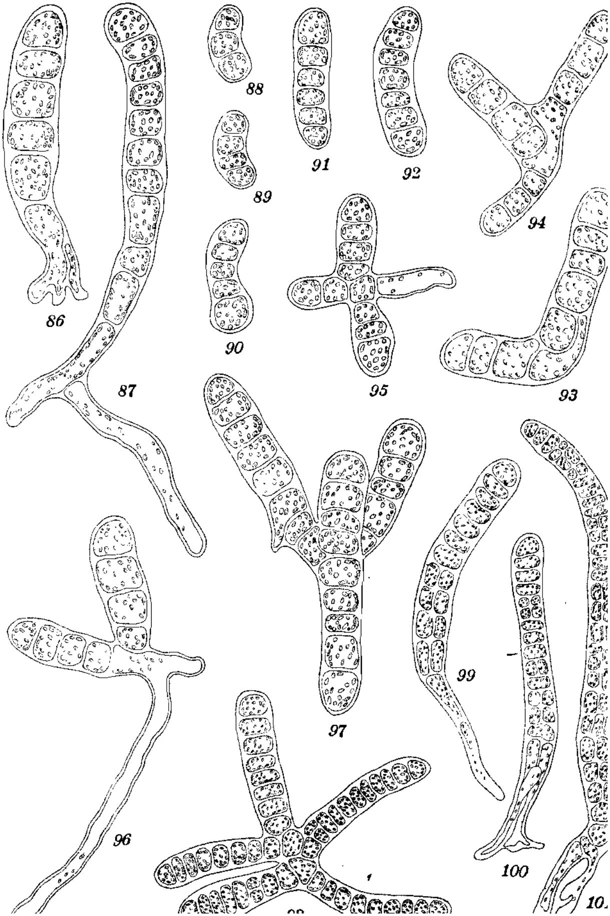
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The Host-Parasite Relationship in Olpidium.

By

Shunsuke Kusano.

With ten Figures in the Text.

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I. Introduction.

Olpidium Viciae Kus. and *O. Trifolii* Schröt. establish the intracellular development on the epidermis of the host plant. The development proceeds very rapidly, being completed in a week under favourable conditions. The

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mature gametangium germinates within half an hour and the infection soon follows. On account of these characteristics the process of invasion and the developmental stages of the fungus can be followed out in the living condition with great ease and clearness. Therefore, these fungi were chosen as the material for the investigation of the relation of host and parasite, and on carrying out inoculation experiments their parasitic nature has been examined on some eighty species of Phanerogams, comprised in families very divergent in relationship. In addition the problem has been approached by studying at some length the chemotaxis of their swarmcells. There remains much to be done. Yet the results so far obtained appear to throw some light upon fundamental problems underlying the principle of phytopathology, so that they will be given in the form of the present report.

As the object of the present work is to make a general survey on the host-parasite relationship and much time has been taken to carry out inoculation experiments with plants as numerous as possible, an intimate relation between each plant and the parasite has not been studied satisfactorily. To yield a conclusive result along this line a cytological study on the inoculated materials with the aid of microtome and physiological experiments on the parasite are much to be desired. They shall be reserved for future communications. Moreover, the varietal relation of the examined cultivated plants to the parasite has been neglected, one or two varieties being taken as representing the species. On the part of the parasite no special attention was paid as to the existence of strains which might reveal different relations to the same host plant. The material of *O. Viciae* was originated from a diseased stock of *Vicia unijuga* brought from the field near Tokyo and planted in the laboratory ground some years before. The material of *O. Trifolii* was obtained at desired times from different places in the laboratory ground.

Thanks are due to Prof. K. Aso of our Faculty, who generously supplied me with the material of the leguminous plants cultivated in his laboratory.

II. Infection of the Leguminosae.

1. Internal Tissue.

In a study of *Olpidium Trifolii* (Kusano, 1929 a) the infection of extra-epidermal cells of the proper host, *Trifolium repens*, has briefly been reported. The method of testing it is very simple. Introducing longitud-

inal sections of the stem, the petiole, and the mid-rib of the blade in a hanging drop of water, in which the swarmcells[1] are found suspended, the latter are soon attracted to the surface of the sections and on the next day spherical fungus bodies are found embedded in the protoplast of the parenchymatous cells. It appears that the infection of young internal cells takes place more readily than that of the epidermis. In the present study this method is adopted to determine whether a given plant is susceptible or not. As an advantage attached to this method, we can follow out the developmental features of each fungus body through the microscopic examination at desired intervals of time. Under favourable conditions the sections remain alive safely more than a week, during which interval the development of the gametangium may often be completed.

At first the infection was tested with *O. Viciae* on the internal tissue of its own host. As in the case of *O. Trifolii*, a fairly good result has been obtained. Next, other species of the genera, to which the hosts of these fungi belong, were examined and they also were found to be easily infected.

Further, having found that *O. Viciae* and *O. Trifolii* can invade their reciprocal hosts as profusely as their own hosts, the experiments were extended to a number of available species of the Leguminosae, wild and cultivated. The sections to be inoculated were taken from young stems or petioles, as they generally furnish large thin-walled parenchymatous cells, revealing a clear internal structure under a microscope. Those from older portions, consisting of cells whose wall undergoes more or less thickening, were proved to be less susceptible to infection. The tissue of too young portions was not used, since the internal view of the cell is obscured by its dense content. In arboreous plants the sections from the seedlings gave a better result of inoculation than those from the young shoots.

Beyond our expectation most species tested were infected by both fungi, as will be seen in the accompanying table (Table 1).

1) Gametes and planozygotes behave quite similarly as regards the infection, so that they are conveniently denoted by the word swarmcells.

TABLE 1.

Showing the result of inoculation with *O. Viciae* and *O. Trifolii* on the internal tissue and epidermis of the Leguminosae. +, infection positive; —, infection negative. Arboreous plants are marked with *.

Plant	*O. Viciae*		*O. Trifolii*	
	Internal tissue	Epidermis	Internal tissue	Epidermis
Albizzia Julibrissin Durraz.*	—		—	
Amorpha fruticosa L.*	+		+	
Arachis hypogaea L.	+		+	
Astragalus sinicus L.	+	+	+	+
Caesalpinia japonica S. et Z.*	—		—	
Caragana Chamlagu Lam.*	+		+	
Cassia mimosoides L. var. *nomame* Mak.	—		—	—
C. occidentalis L.	—		—	
Cercis chinensis Bunge*	+		+	
Desmodium japonicum Miq.	—		—	
Falcata japonica Komar.	+		+	
Gleditschia horrida Mak.*	—		—	
Glycine Soja Benth.	+	+	+	+
Kraunhia floribunda Taub. var. *typica* Mak.*	+ (Root +)		+ (Root +)	
Lathyrus maritimus Bigel.	+		+	
L. latifolius L.	+		+	
Lespedeza juncea Pers. var. *sericea* Maxim.				+
Lotus corniculatus L. var. *japonicus* Regel.	+		+	
Lupinus luteus L.	+		+	
Medicago minima Lamk.	+		+	
M. sativa L.	+		+	
Meliotus officinale Willd.	+		+	
Mimosa myriophylla Bong.	—		—	
Ornithopus sativus Brot.	+		+	
Phaseolus angularis Wight.	+ (Root +)	+	+ (Root +)	+
P. vulgaris L.	+	+	+	+
Pisum sativum L.	+ (Root +)	+	+ (Root +)	+
Pueraria triloba Mak.	+		+	
Robinia pseudacacia L.*	+		+	
Sophora japonica L.*	+ (Root +)		+ (Root +)	

TABLE I. (*Continued*)

Plant	*O. Viciae*		*O. Trifolii*	
	Internal tissue	Epidermis	Internal tissue	Epidermis
Trifolium incarnatum L.	+		+	+
T. medium L.	+		+	
T. pratense L.	+	+	+	+
T. repens L.	+ (Root +)	+	+ (Root +)	+
Vicia Faba L.	+ (Root +)	+	+ (Root +)	+
V. hirsuta Koch.	+		+	
V. sativa L.	+	+	+	+
V. sativa L. var. *angustifolia* L.	+	+	+	+
V. unijuga Al. Br.	+ (Root +)	+	+ (Root +)	+
Vigna Catiang Endl. var. *sinensis* King.	+	+	+	+

In the inoculated sections from various organs (stem, petiole, and vein of the blade) any young parenchymatous cell, such as a green cortical cell, hyaline pith cell, and smaller meristematic cell in the vascular bundle, may be infected with equal ease.

When several individuals of the fungus gain entrance in a single large cell, they are drawn together close to the host nucleus. Also chloroplasts or starch grains are aggregated round them (Fig. 1). For a few days all these bodies exist in a conglomerate mass which becomes more and more prominent owing to the growth of each fungus body. Hereafter, the fungus bodies are separated from each other and distributed freely in the cell cavity. The host nucleus may often stand apart from any of them.

In general the large cell is subject to a heavy infection. For instance, in *Vicia Faba* and *V. sativa* var. *angustifolia* a host cell is often found to contain twenty to thirty and in extreme case more than fifty fungus individuals. Most of them, however, appear to perish in the course of the subsequent development.

As a matter of fact the host cell in the piece of tissue, being interrupted of nutritive resources, is not able to supply the residing fungus with a sufficient amount of food. The fungus is therefore much stunted in growth, but its development into the gametangium or resting cell may proceed at the usual rate, so that under favourable conditions the mature

gametangia are obtained on the eighth or ninth day after infection. In a large host cell they lie freely in the cell cavity in a spherical form. Before germination they are a little pointed at one side and form exit beaks to liberate the gametes in the cavity of the host cell. Some gametangia may become pear-shaped and their pointed end comes often in contact with the wall of the host cell. I missed their germination; it would appear that there is formed the exit beak which, perforating the wall as usual, makes the gametes liberate outside the host cell.

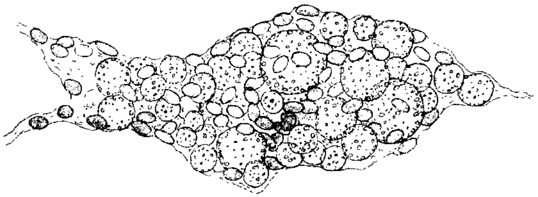

Fig. 1. Aggregate of young fungus bodies (more than 30) of *Olpidium Viciae* in the protoplast of a green cortical cell in the stem of *Vicia Faba* (6 days after inoculation). ×800

Infection of the root parenchyma by both fungi was tested in *Kraunhia floribunda, Phaseolus angularis, Pisum sativum, Sophora japonica, Trifolium repens, Vicia Faba,* and *V. unijuga,* giving a positive result in all. As long as the tissue remained alive, the growth of the invader was taking place.

In every species of plants the host cell never undergoes such change in dimension or form as is observed on the intact proper host, looking like the uninvaded cells, nor do its surrounding cells show any histological change.

Among the plants so far tested, a few, as shown in Table 1, gave the negative result of inoculation. As to how it is brought about an account will be given subsequently to the prosecution of further experiments.

From the above experiments we learn that *Olpidium Viciae* and *O. Trifolii* are capable of attacking the parenchyma of various plants included in the Leguminosae. The degree of infection appears to be essentially similar throughout.

The development of the fungus into an adult form is not ascertained in all susceptible plants, since in many cases the inoculated sections are not

kept alive for the desired interval of time. Judged from the general feature of growth it may be predicted that in most plants, if not all, the normal parasitic development would be possible.

2. Wound.

In a small piece of the host tissue, interrupted of the nutritive connection with the entire plant, the normal growth of the fungus and the normal reaction of the host cell are not expected. Deficiency of nutriment seems to exert a great influence upon the activity of both the fungus and the host cell. To eliminate this defect the inoculation has been tried on the wound surface of the young shoot. On removing the epidermis, sometimes together with the underlying cortical tissue, the thin-walled parenchymatous cells are exposed to some extent. After washing the wound surface with water the germinative gametangia were directly applied on the wound. If protected from drought, the exposed cells on the wound may retain their vitality for two or more weeks.

(1) *O. Viciae* and *O. Trifolii* on their respective hosts. The infection of the wound by both fungi and its effect do not reveal any difference from the epidermal infection and its effect. When the wound tissue is yet young and vigorous, the cells surrounding the host cell undergo multiplication resulting in a prominent tumour. Otherwise, as often observed in the case of the epidermal infection, the host cell and adjoining cells undergo no histological change, even though the gametangia or resting cells are growing in aggregate on the wound surface.

(2) *O. Viciae* and *O. Trifolii* on *Vicia Faba*. On the sixth day *O. Viciae* causes here and there on the surface of the wound small swellings, which grow on the ninth day into apparent tumours of irregular forms. Towards the fourteenth day the tumour is growing larger and the growth may c ntinue till the seventeenth day.

The tumour is evidently produced as an effect of the reaction of the host tissue to the fungus. In the centre of the tumour one to several invaded cells are found, in which the development of the fungus proceeds normally.

The formation of the tumour is not pronounced, if the invader develops into the resting cell.

Infection of the wound by *O. Trifolii* is obtained as profusely as by *O. Viciae*. But the histological reaction of the host is almost negligible; the wound surface may be a little swollen at the spot where the infection

takes place, yet no tumour is produced. Notwithstanding that, the development of the gametangia proceeds normally and the gametes are liberated at the usual period.

(3) *O. Viciae* on *Trifolium repens*. The wound surface of the young petiole shows the infection, though its degree is less than on the proper host, *Vicia unijuga*. Usually the histological reaction is not observed, but rarely multiplication of cells takes place around the host cell. The gametangia come to maturity at the usual rate.

(4) *O. Trifolii* on *Vicia unijuga*. The infection takes place readily. A number of the gametangia and resting cells are developed in rows in several cells without inducing any histological change to the wound surface. The host cell often begins to turn brown earlier than usual, that is, previous to the maturation of gametangia or resting cells. On the eighth day the gametangia attain maturity and liberate the gametes on giving water.

(5) *O. Viciae* and *O. Trifolii* on *Pisum sativum*. *O. Viciae* produces tumours on the wound surface as on *Vicia Faba*, but *O. Trifolii* gives no external sign of infection, though under a microscope some cells on the wound surface are found to contain the gametangia which advance in development ready to produce the gametes.

(6) *O. Viciae* and *O. Trifolii* on *Phaseolus angularis*. The cavity of the invaded cells turns brown, while the gametangia are still going to grow. This colouration indicates a process of disorganization of the host cell. Some gametangia remain small, being spherical or ovoid, and only attaining the width of the host cell, while some grow so large as to occupy nearly the elongated entire cavity of the host cell. Irrespective of various sizes the gametangia are able to attain maturity and to produce the gametes.

The wound tissue infected by either of both fungi does not undergo any histological change.

(7) *O. Viciae* and *O. Trifolii* on *Vicia sativum* var. *angustifolia*. The infection of the wound tissue by both fungi is readily obtained and the development of the gametangia proceeds normally. In the affected tissue no histological reaction is observed.

(8) Other plants. The wound infection was further tested with *Lupinus luteus*, *Phaseolus vulgaris*, and *Vigna Catiang* var. *sinensis*. The ready infection by both fungi was obtained and their vigorous development took place in all of them. Maturation of gametangia was attained at the usual rate.

The infection of *Ornithopus sativus* by *O. Trifolii* and of the seedlings

of *Sophora japonica* by *O. Viciae* were also successful and the mature gametangia were obtained.

As has been expected, the fungi developing in the internal cells exposed to the wound surface generally grow larger than in those on a piece of tissue. It is evidently due to the fact that the host cell on the wound is supplied with food substances just as the invaded epidermal cell on the intact plant body.

Further experiments with other plants were not carried out. But from the results given above it may be predicted that the plants, whose isolated tissues are infected and enable the invader to grow, would mostly, if not all, allow the invader of the complete development on their wound surface.

3. Epidermis.

From the preceding experiments we see that young extra-epidermal cells in most of the leguminous plants tested are easily susceptible to both *O. Viciae* and *O. Trifolii*. In the degree of infection these cells show no remarkable difference. From this fact it is strongly expected that the epidermis of these plants would also be susceptible. If not, the difference between the epidermal and extra-epidermal cells in this respect would offer a subject of study. To elucidate the matter the inoculation has been done on the epidermis of seedlings or young shoots of several plants, in which the infection of the extra-epidermal cells has already been ascertained (see Table 1).

(1) *O. Viciae* and *O. Trifolii* on their reciprocal host plants. As far as the macroscopic observation extends, *O. Viciae* and *O. Trifolii* do not give any sign of infection on their reciprocal hosts, that is, *O. Viciae* on *Trifolium repens* and *O. Trifolii* on *Vicia unijuga*. On this account I stated in a previous paper (Kusano, 1929 a) that both fungi are not able to attack their reciprocal hosts. As this fact seems to contradict the result of the inoculation on the internal tissue, attention has been devoted in the present study to the microscopic examination of the epidermis of the inoculated plant. Examining some days after inoculation the removed fresh epidermis under a microscope we find that some cells in *Trifolium repens* inoculated with *O. Viciae* and in *Vicia unijuga* with *O. Trifolii* contain hyaline globular fungus bodies, being in the course of the normal growth. The invaded cells show no deviation in form and size from the uninvaded ones. In consequence the fungi do not attain the size so large as those occurring in the much enlarging host cell of the proper host. Although

they thus remain smaller than usual, they are able to establish a complete development.

In general the degree of infection of the epidermis by both fungi is less in their reciprocal hosts than in their respective hosts. Furthermore, the development of the fungi is not so vigorous as on the wound surface. In addition it happens that the host cell of either *O. Viciae* or *O. Trifolii* turns brown while the fungi are yet growing. As a whole the epidermal cell is, as a host cell, far inferior to the internal cell. Since the difference in thickness in the outer wall of the young epidermal cell in both plants, if it exists, is not concerned with the difference in the degree of infection, we reasonably infer that any other morphological character or some internal physiological condition of the epidermal cells are responsible for a less percentage of infection. As previously reported, both fungi do not show any difference in the degree of infection of the internal tissue of either *Trifolium* or *Vicia*. It is likely that in each plant the epidermal and internal cells present a certain difference in the physiological condition in regard to the invasion of the fungus. To elucidate the matter, however, a further investigation is required.

The result of experiments is sufficient to draw the conclusion that *O. Viciae* and *O. Trifolii* are able to invade the young epidermal cells of their reciprocal hosts and to develop therein. However, they do not cause the typical histological effect and the percentage of infection is less than in their proper hosts.

(2) *O. Viciae* and *O. Trifolii* on *Vicia Faba* and *Pisum sativum*. The inoculation with *O. Viciae* on the seedlings or young shoots of *Vicia Faba* and *Pisum sativum* yields a remarkable result. Infection is obtained as good as of its proper host (*V. unijuga*) and the symptom of the resulting disease is quite similar or rather more conspicuous (Fig. 2, *A*, *B*, *C*). The tumour cells around the host cell grow considerably larger and elongating upwards form together a conical process carrying the brown corrupted host cell on its apex. On the whole the reaction of these plants to the parasite is more pronounced than of the proper host, and the heavily infected leaves undergo a great distortion and often curling.

To observe the feature of the field infection of these plants by *O. Viciae*, their seeds were planted in April on the ground where the parasite was spreading upon the young shoots of the proper host. The seedlings were readily affected and the diseased tumours were found on the lower portion of the stem and young leaves from the lower nodes. The infection appeared to have been caused both by the resting cells remaining dormant

in the soil and by the gametangia developing upon the proper host.

Thus it is ascertained that *O. Viciae* usually occurring on a wild herb, *Vicia unijuga*, may be injurious to the cultivated plants, although no record has yet been given. In great probability an association of the parasite and these plants is not established in the cultivated soil. However, if the soil is once infested with the resting cells, the disease would possibly prevail on these plants cultivated there.

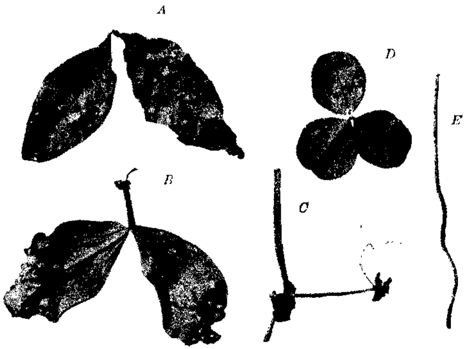

Fig. 2. Photograph showing the typical symptom of disease caused by *Olpidum Viciae* and *O. Trifolii*. *A*, *O. Viciae* on *Vicia unijuga*; *B*, *O. Viciae* on *Vicia Faba*; *C*, *O. Viciae* on *Pisum sativum*; *D*, *E*, *O. Trifolii* on *Trifolium repens*.

Otherwise is the result of inoculation with *O. Trifolii*. Infection may take place equally well, but an apparent disease is not caused. While the gametangia are about to mature, small necrotic spots appear on the inoculated surface. The formation of these spots is assigned to the disintegration of the invaded cell and the cells just surrounding it. It may be remarked that these spots are by no means characteristic to the infection by *O. Trifolii*, since *O. Viciae* also causes similar spots on the surface of the tumour. The invaded cell as well as the surrounding cells remain almost normal in size and form (Fig. 3). On this account the affected plant is apparently healthy, leading us to a mistaken conclusion that the plant is absolutely

resistant to the attack of *O. Trifolii.*

Complete development of the gametangia is not always assured. When the necrosis of the host cell takes place earlier than usual, they appear to perish without attaining maturity.

(3) *O. Trifolii* on *Trifolium pratense* and *T. incarnatum*. It has previously been remarked that *Trifolium pratense* growing in the field in close approximation with *T. repens* affected by *O. Trifolii* remains free from the same parasite (KUSANO, 1929 a). Confirming in the present work the infection of the extra-epidermal tissue of that plant, it becomes clear that the result of the field observation given above is not decisive in proving the resistance. In trying the inoculation I find that the young portion of the shoot is tightly inclosed in the membranaceous stipule. When the inoculum is directly applied on the epidermis of the shoot after removing the stipule, the infection is obtained without difficulty. Remarkably enough, the development of the invader and the formation of the typical tumour proceed just as on *Trifolium repens.* It is concluded that *T. pratense* is a plant susceptible to the attack of *O. Trifolii.* We now come to a belief that its non-infection in nature is ascribed to inaccessibility of the fungus to the young epidermis.

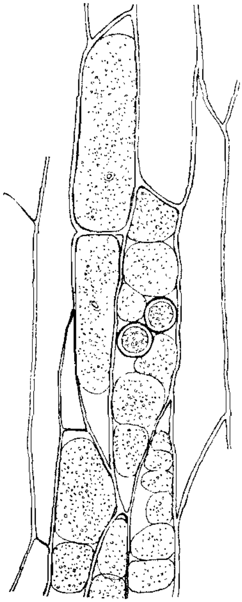

Fig. 3. *Olpidium Trifolii* on *Pisum sativum* (7-day stage). The invaded epidermal cells remain undeformed. ×300

In *Trifolium repens* the apex of the creeping stem lies close to the surface of the ground and thus is ready to receive the swarmcells suspended in rain water which flows down from the upright diseased leaves. On the other hand *T. pratense* grows erect and the apex of the shoot attains higher and higher above the ground, being subject to drought. While in *T. repens* the bud leaves are soon exposed from the membranaceous

stipules and are well wettable, in *T. pratense* the young leaves are remaining for a long period compactly inclosed in the large stipule arisen from the node just below and in addition are densely covered with hairs, making the surface unwettable (Fig. 4). It will be seen from these facts that it is not easy for the parasite to gain access to the young epidermis of *T. pratense*.

Here we recall the fact that in Europe *T. pratense* is reported to be affected by a parasite which is identified with either *Synchytrium Trifolii* Pass., *Olpidium Trifolii* (Pass.) Schröt., or *Urophlyctis Trifolii* (Pass.) P. Magn. (cf. KUSANO, 1929 a). There is some doubt about the identification of the parasite. Whatever genus it may be, the parasite requires a film of water over the surface of the host to effect the infection by the swarmcells. From the characters of the host plant mentioned above it would appear that the occurrence of the problematic parasite on this plant is by no means very common in Europe. It is supposed that the swarmcells liberated in spring in the soil from the resting cells may possibly invade young shoots still lying underground.

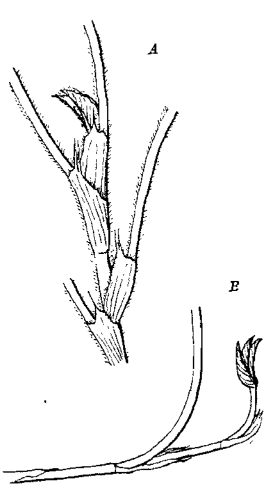

Fig. 4. *Trifolium pratense* (*A*) and *T. repens* (*B*) showing the apical portion of the stem.
Nat. size.

Whether this may be the fact or not, a wintering stock of this plant was transplanted in early spring to a pot and being covered with a layer of sawdust was kept under moist conditions. The sprouted young leaves were hygrophilous and less tomentose. Over them the affected leaves of *Trifolium repens* bearing just matured gametangia were laid, taking the precaution to keep the plant surface sufficiently wetted for several days. Observed macroscopically, no sign of affection was indicated on the growing leaves, but a microscopic examination of the removed epidermis showed that the growing fungus bodies exist in several cells. Owing perhaps to a less vigo-

rous growth of the plant the invaded cells and those surrounding them did not react to undergo any deformation, though the fungus development proceeded to give rise to mature gametangia.

T. incarnatum, like *T. pratense*, grows upright and is provided with the membranaceous large stipules covering the densely tomentose primordial leaves for a long time. As the surface of this plant is unwettable, the swarmcells are not, under the natural condition, accessible to the epidermis. However, on the artificial inoculation the infection is obtained on the young portion. The host cell is enlarged and the tumour is produced as on *T. repens*.

(4) *O. Viciae* and *O. Trifolii* on other plants. From these species of the Leguminosae, the internal tissue of which has been ascertained to be infected, the following plants were chosen for further inoculation experiments:

Vicia sativa.
V. sativa var. *angustifolia*.
Phaseolus vulgaris.
P. angularis.
Glycine Soja.
Astragalus sinicus.
Vigna Catiang var. *sinensis*.

As the first trial the seeds of *Phaseolus vulgaris*, *P. angularis*, and *Glycine Soja* were planted together with those of *Vicia Faba* and *Pisum sativum* in the soil where *Vicia unijuga* was growing seriously affected by *O. Viciae*. At due times their seedlings grew up among the diseased individuals of *Vicia unijuga*. While, as already mentioned, *Vicia Faba* and *Pisum sativum* were presenting a visible symptom of the disease, the other plants did not give any sign of infection to be recognized macroscopically. It was not clear whether they were non-susceptible or susceptible.

To elucidate the matter by closer microscopic examination, the inoculation was made on the potted seedlings or on young shoots standing in water. After the gametangia were applied on a definite portion of the young plants, the daily examination was made sometimes on the inoculated portion with the aid of a hand lens and at other times on the removed epidermis under a microscope. On the external appearance all these inoculated plants were entirely healthy, but in fact they were infected by both fungi as mentioned below.

Phaseolus vulgaris: Examined after six days some epidermal cells, often turning brown, contain the gametangia or resting cells nearly full

grown. The host cell of the gametangium is sometimes found a little swollen and the surrounding cells produce one or two septa. We see therefore that the host plant shows a slight histological reaction to the invasion of both *O. Viciae* and *O. Trifolii.*

Glycine Soja: A similar result is obtained. At the same period as before, small necrotic spots are recognized. The spots are represented by the host cells containing nearly adult gametangia or resting cells.

Phaseolus angularis: The feature of infection is essentially the same. As an interesting fact the necrotic spots appear somewhat earlier, just as observed in the infection of the wound surface. The brown host cell contains the growing gametangia. It appears probable that the fungus may give to the host cell a detrimental effect and consequently its own development may be more or less interfered with. As showing an early stage of necrosis the cytoplasm in the hyaline host cell is found disintegrated into smaller or larger globules, while the matrix of the nucleus and also of the fungus body is provided with globules or is broken down into structureless masses of granules and globules. The necrotic process is not observed in the host cell containing a small fungus body. It appears to show that the detrimental action of a less vigorous fungus is feeble. From this fact we see that some host cells perish early without allowing the fungus complete development, while some maintain their activity until the fungus comes to maturity.

The host cells always remain normal in size and the surrounding cells undergo almost no morphological change.

The brownish colouration of the host cell in *Phaseolus vulgaris, P. angularis,* and *Glycine Soja* points out that a more or less disturbance is caused in the metabolic action of the invaded cells. In *P. angularis* the disturbance sometimes advances so far as to cause the disintegration of the protoplast, leading the growing fungus to death. Hence this plant does not seem to be a favourable host.

Vicia sativa: Inoculation on young shoots gives a positive result of infection. With *O. Trifolii* necrotic spots are recognized, where the infection takes place. The host cell as well as the surrounding cells are a little enlarged. Maturation of gametangia is attained in nine days after inoculation.

The infection by *O. Viciae* is essentially similar. A little enlargement of the host cell takes place. At the point of infection a slender stem may be caused to bend slightly. The cells surrounding the base of trichomes appear to be infected more readily. Gametangia mature at a usual period.

Vicia sativa var. *angustifolia*: The young epidermis is easily infected by both fungi, but in the infected spot no anatomical change is induced. In one case the infection of cells surrounding the base of a trichome (Fig. 5, *B*, *C*) gives rise to a small protuberance. Sometimes the gametangia

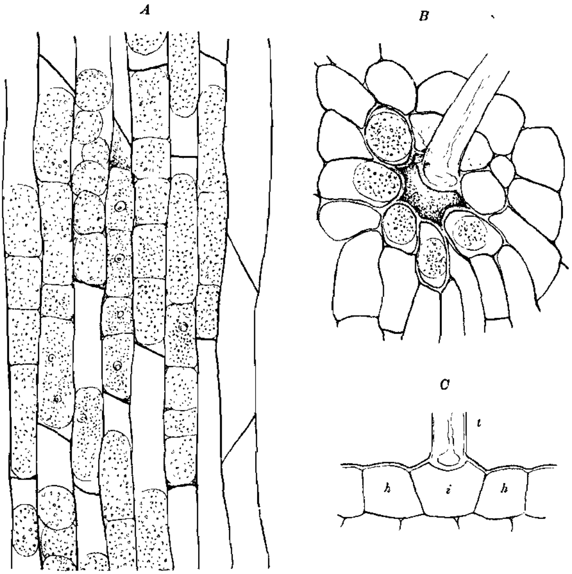

Fig. 5. Infection of epidermis. *A*, *Astragalus sinicus*, in which the epidermis of the hypocotyl is heavily infected by *Olpidium Trifolii*; *B*, *Vicia sativa* var. *angustifolia*, in which the epidermal cells are invaded by *O. Viciae* through the dead basal cell of a trichome; *C*, diagram of the same showing the relation of the invaded cells (*h*) to the trichome (*t*) and its basal cell (*i*). ×300

grow vigorously, occupying almost the entire cavity of the host cell. They liberate the gametes at a due time when water is given.

Astragalus sinicus: Very heavy infection of the hypocotyl is obtained by both fungi. Gametangia proceed to mature without causing any histological change on the affected portion of the host (Fig. 5, *A*).

Vigna Catiang var. *sinensis*: The hypocotyl of seedlings is easily infected. Both fungi may cause the brownish discolouration to the host cell. In the host cells having undergone such change some individuals of *O. Viciae* are disorganizing but others may grow larger into mature gametangia. The infection by *O. Trifolii* gives rise to an essentially similar effect. Sometimes the surface of the stem is a little swollen due to an increase in number of the slightly hypertophied cells.

We have thus ascertained the epidermal infection in eleven plants by *O. Viciae* and in thirteen plants by *O. Trifolii*, excluding the proper host (Table 1). *The results of experiments so far obtained show that the* plants, internal cells of which are susceptible, may be subject to the epidermal infection. However, their reaction presents a certain variation; some produce the typical tumour as do *Vicia Faba* and *Pisum sativum* attacked by *O. Viciae* and *Trifolium pratense* and *T. incarnatum* attacked by *O. Trifolii*. In this respect the epidermal and extra-epidermal (wound) infection give the same effect. Generally the degree of infection of the epidermis is less than that of the internal tissue. How this difference is brought about will be considered later on.

Looking over the results of a series of experiments so far made we see that the host relation of *Olpidium* to a plant may be understood as the host relation to individual cells composing the entire plant body. Since the fungus develops in the living plasm, only the parenchymatous or meristematic cells are subject to infection. It is concluded that, though the fungus occurs in nature only in the epidermal cell, it relates similarly to other cells of the leaf, stem, and root. Therefore, with respect to the host relation *Olpidium* shows a great divergence from *Synchytrium* which establishes a similar parasitic life. For instance, *S. fulgens* on *Oenothera* invades exclusively the epidermal cells of the aerial vegetative organs, never attacking those of the root and all other parenchymatous cells (KUSANO, 1929 b). It would follow from this that while *Synchytrium* perceives a difference between the epidermal and other living cells of *Oenothera* regarding the chemical or physical natures, almost all living cells of many recognized host plants appear to be alike to *Olpidium*, however they may show in themselves a great variation in chemical and other natures.

III. Infection of other Families.

It is entirely beyond our expectation that the two forms of *Olpidium*, each occurring, as far as known, on a single species of the Leguminosae, are able to be parasitic on a large number of species in the same family and it is surprising that numerous other families of Phanerogams are also equally infectious.

Early in the course of the present work the internal tissues of some plants not belonging to the Leguminosae were once submitted to an examination of infection. Unfortunately, the inoculation on these plants failed to obtain the positive result, so that a further trial has not been attempted. Later, on examining another plant incidentally inoculated I was struck with the fact that its internal tissue is infected with great ease. Interested with this find my attention was first called to the possibility of infection of the non-leguminous plants. Of innumerable species that were accessible some forty comprised in several families have been examined up to the present. Awaiting more extensive and intensive studies for the detailed account I may give in the following the results so far obtained[1].

Using the small sections of young parenchymatous tissues the infection was ascertained in the majority of plants examined. The results of inoculation are given in Table 2. The developmental feature of the entered fungus was not studied accurately on each plant, but in most cases the daily growth was taking place, as far as the observation during first two to four days extended. It is almost certain that in some of the invaded plants[2] the development of the fungus was inhibited, seemingly on account of the uncongeniality of the host plasm with the invader (compare the results of the wound infection given below). As to the negative result of inoculation in some plants, as mentioned in Table 2, it is not yet conclusive whether the resistant character of the plant or some defect of the experiment is responsible. About this point we will consider again on the basis of further experiments.

TABLE 2.

Showing the result of inoculation on the internal cells of non-

1) Since the infectivity of both fungi upon the same plant, as mentioned in the preceding experiments, is essentially similar, attempt has not been made to carry out the parallel experiments with both fungi.

2) In Table 2 these plants are marked with *.

leguminous plants. *OV*, *Olpidium Viciae*; *OT*, *O. Trifolii*; +, infection successful; —, no infection.

(Plant)	(Fungus)	(Infection)
Araceae		
Pinellia ternata Breitenb	OT	+
Balsaminaceae		
Impatiens Balsamina L.	OV	+*
Campanulaceae		
Stellaria media Cyr	OT	+
Chenopodiaceae		
Chenopodium album L. var. *centrorubrum* Mak.	OT	+*
Spinacia oleracea L.	OT	+
Commelinaceae		
Commelina communis L.	OV, OT	+
Compositae		
Chrysanthemum sinense Sab.	OV	+?
Dahlia variabilis Desf.	OV	—
Lactuca Scariola L. var. *sativa* Bisch.	OV, OT	—
Petasites japonicus Miq.	OT	—
Taraxacum Platycarpum Dahlst	OV, OT	+*
Convolvulaceae		
Calystegia japonica Choisy.	OV	—
Crassulaceae		
Sedum alboroseum Bak.	OV	+
Cruciferae		
Brassica chinensis L. var. *oleifera* Mak.	OT	+
B. oleracea L. var. *capitata* L.	OT	+
B. chinensis L.	OV, OT	+
Raphanus sativus L.	OV	+
Roripa Nasturtium-aquaticum Hayek	OV, OT	+
Cucurbitaceae		
Cucumis sativus L.	OV, OT	+
Cucurbita moschata Duch. var. *melonaeformis* Mak.	OV	+
Liliaceae		
Hosta Sieboldiana Engl.	OV	—
Tulipa Gesneriana L.	OV, OT	+
Oenotheraceae		
Oenothera biennis L.	OT	—
O. Hookeri Terr. et Gray.	OT	—
Oxalidaceae		
Oxalis corniculata L.	OT	+
O. violacea L.	OV, OT	+
Plantaginaceae		
Plantago major L. var. *asiatica* Decne	OV	—
Polygonaceae		
Fagopyrum esculentum, Moench.	OV, OT	+
Polygonum Blumei Meisn.	OT	+
P. orientale L. var. *pilosum* Meisn.	OV, OT	+
Rumex Acetosa L.	OT	+
R. Acetosella L.	OT	+
R. japonicus Meisn.	OV, OT	+

TABLE 2. (*Continued*)

(Plant)	(Fungus)	(Infection)
Ranunculaceae		
Ranunculus japonicus Langsd.	OV, OT	+*
R. ternatus Thunb.	OT	+*
Rosaceae		
Rubus trifidus Thunb.	OV	−
Solanaceae		
Lycopersicum esculentum Mill.	OV	−
Physalis Alkekengi L.	OV	+*
Solanum Melongena L. var. *esculentum* Nees.	OV	+
S. tuberosum L.	OT	+*
Vitaceae		
Cissus japonica Willd.	OT	+*

The reaction of *Chrysanthemum sinense* to the invasion of *O. Viciae* is perhaps quite unique. Although numerous cysts occur on the surface of several cells, no fungus body is detected in the protoplast of these cells. Our attention, however, is attracted by the occurrence of glistening pro-

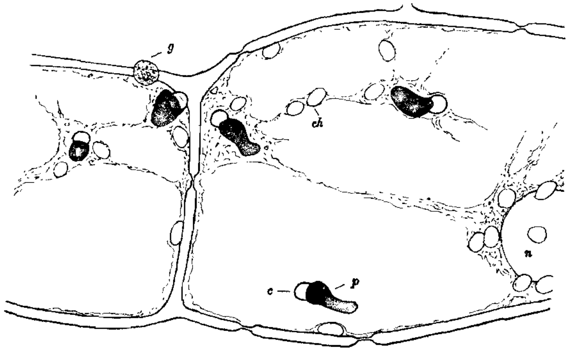

Fig. 6. Green cells of *Chrysanthemum sinense* inoculated with *Olpidium Viciae*. *p*, process; *c*, empty cyst; *g*, encysted zygote; *ch*, chloroplast; *n*, host nucleus. ×800

cesses arising from the inner surface of the cell wall (Fig. 6, *p*). Being homogeneous in appearance their inner structure is not clearly shown in

the fresh condition. On the outer surface of the wall just at the point where the process arises we find an empty cyst (*c*), showing that the fungus plasm gains entrance into the cell. On this account the process may be in its nature similar to the sheath often found in the haustoria of powdery mildews, smuts, and rusts (RICE, 1927, p. 85) and to what is termed the callosity caused by penetrating hyphae of several facultative parasites (YOUNG, 1926), by means of which the host cell is considered to stop the invasion of the fungus. Likewise the process seems to inclose the invading fungus and to prevent its advance into the host plasm. Whether the material of the process is originated from the cell wall or newly laid down by the host plasm is not decided. At any rate the formation of the process may be regarded as a reaction of the cell to the invasion of the fungus. This reaction is of great significance, because it results in an "active resistance," to which FISCHER-GÄUMANN (1927) attempt to apply the conception of immunity.

To obtain a more accurate knowledge of the developmental feature of the fungus after infection the inoculation has been made on the wound surface of some of the plants used in the preceding experiments. Since in this experiment the wound surface was examined a few days after inoculation, attention has not been given to decide whether the absence of any fungus body on the inoculated surface is the result of non-infection or of disintegration of the fungus body after infection. The plants used and the result obtained are given in the following table (Table 3).

TABLE 3.

Showing the result of inoculation on the wound surface.

(Plant)	(Fungus)	(Result)
Balsaminaceae.		
Impatiens Balsamina.	*OV, OT*	Fungus not found.
Campanulaceae.		
Stellaria media.	*OV*	Development complete.
Chenopodiaceae.		
Chenopodium album var. centrorubrum.	*OT*	Fungus not found.
Compositae.		
Chrysanthemum sinense.	*OV*	Fungus not found.
Dahlia variabilis.	*OV*	Fungus not found.
Cruciferae.		
Brassica chinensis.	*OT*	Development complete.
B. oleracea.	*OV*	Development not complete.
Rhaphanus sativum.	*OV*	Development not complete.
Roripa Nasturtium-aquaticum.	*OT*	Development not complete.

TABLE 3. (*Continued*)

(Plant)	(Fungus)	(Result)
Cucurbitaceae.		
Cucumis sativus.	*OV*	Development complete.
Cucurbita moschata var. *melonaeformis.*	*OV*	Development complete.
Liliaceae.		
Tulipa Gesnesiana.	*OV*, *OT*	Development not complete.
Oxalidaceae.		
Oxalis violacea.	*OV*, *OT*	Development complete.
Polygonaceae.		
Fagopyrum esculentum	*OV*	Development complete.
Polygonum Blumei.	*OV*	*Fungus not found.*
P. orientale var. *pilosum.*	*OV*, *OT*	Development complete.
Rumex japonicus.	*OT*	Development complete.
Solanaceae.		
Solanum Melongena var. *esculentum.*	*OV*	Development is proceeding on the 4th day.
S. tuberosum.	*OV*	Development not complete.
Physalis Alkekengi.	*OV*	Development not complete.
Lycopersicum esculentum.	*OV*	Wound surface soon collapses.
Vitaceae.		
Cissus japonica.	*OV*	Fungus not found.

Thus among twenty-two species of plants examined the fungus is found proceeding in development on fifteen, being able to attain maturity on eight species (Fig. 7). In the following the feature of the fungus development in some of them is given.

Cucumis: Fungus bodies are found abundantly on the wound. Their development proceeds normally and on the seventh day mature gametangia may be obtained.

Cucurbita: A heavy infection is obtained. The fungus development appears to be slower than on other plants. On the eighth day the fungus bodies are yet relatively small in comparison with the size of the host cell, and some assume an appearance of a young stage, while others contain large globules showing a process of disorganization. Perhaps this plant is not an appropriate host. On the twelfth day we obtain full grown individuals as wide as the host cell and provided with beaks. However, many others are far smaller and in varying sizes.

Oxalis: A ready infection is shown by the fact that a single host cell sometimes contains more than twenty fungus individuals. The rate of their development is highly variable. Some remain small and spherical, while others, attaining the width of the host cell, are growing further in the direction of the long axis of the latter. On the eighth day no beak is yet formed, but some appear to approach the maturation stage.

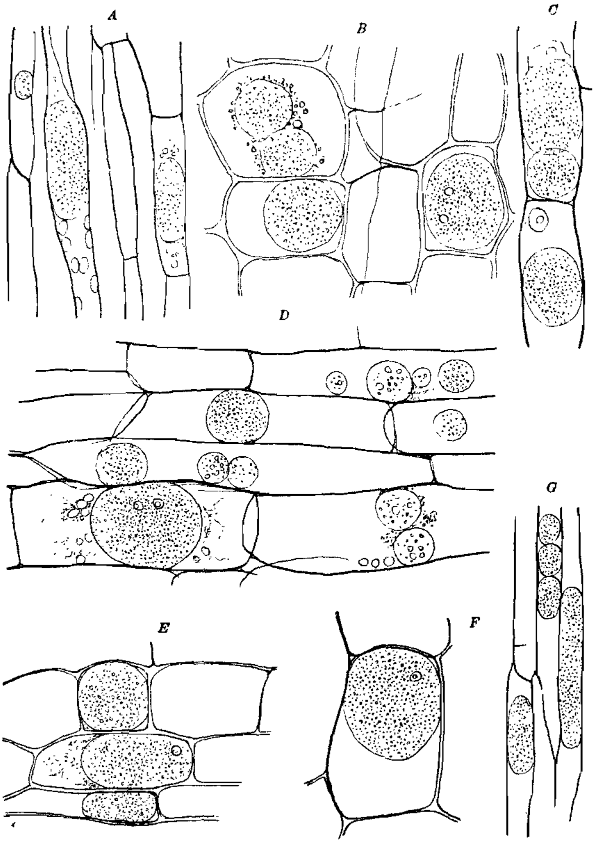

Fig. 7. Infection of the wound surface in several plants. *A*, *Olpidium Viciae* on *Oxalis violacea* (7-day stage); *B*, *O. Viciae* on *Cucumis sativus* (6-day stage); *C*, *O. Viciae* on *Stellaria media* (5-day stage); *D*, *O. Viciae* on *Cucurbita moschata* (12-day stage); *E*, *O. Trifolii* on *Polygonum orientale* (7-day stage); *F*, *O. Trifolii* on *Rumex japonicus* (6-day stage); *G*, *O. Trifolii* on *Brassica chinensis* (4-day stage). ×300

Polygonum orientale and *Fagopyrum esculentum*: A ready infection is obtained and the fungus development proceeds rather rapidly and vigorously. On the sixth day some gametangia occupying a greater part of the cavity of the host cell may attain maturity.

Solanum Melongena: The uppermost cell-layer on the wound is liable to perish soon, rendering the wound surface uniformly brown. Perhaps due to this condition the developing fungus bodies are not abundant. On the fourth day the normally growing individuals produce large globules in their bodies; perhaps they are going to disintegrate.

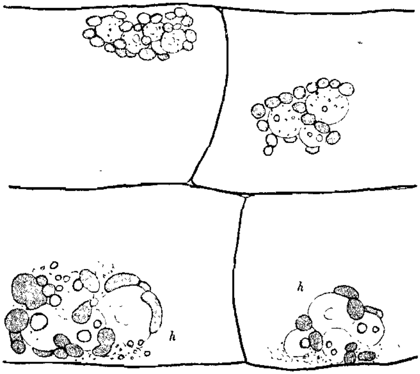

Fig. 8. *Olpidium Trifolii* on the wound surface of the potato stem. *h*, nucleus of the host cell (2 days after inoculation). ×800

Regarding the plants, on which the fungus is not found or its development is not complete, further studies are required to yield the conclusive result about their real relation to the fungus. In *Lycopersicum*, *Chrysanthemum*, and *Dahlia* the infection of the internal tissue has been unsuccessful, so that the absence of the fungus on the inoculated wound may confirm the non-susceptibility of these plants. The internal tissue of the remaining plants, viz. *Impatiens*, *Chenopodium*, *Roripa*, *Brassica oleracea*, *Rhaphanus*, *Polygonum Blumei*, *Solanum tuberosum*, and *Cissus*, has been ascertained to be infected (see Table 2), so that there is a great probability of the infect-

ion of their wounds. In some of them, if not all, the protoplast may be uncongenial with that of the fungus and, being disorganized sooner or later, *causes the death of the fungus.* In potato such a process is clearly demonstrated. Examined two days after inoculation the fungus bodies exhibit a series of the disorganization process (Fig. 8). In some host cells the somewhat grown fungus bodies appear quite healthy, in others they contain large oily globules, and in still others the body loses a sharp outline with the progressive diminution of the granular contents. Also in *Impatiens* the fungus bodies follow a similar fate. Unlike those in potato they seem to undergo disorganization without any growth after infection. The protoplast of the host cell shrinks into an irregular mass, being stained uniformly with chlorophyll diffused out of the disintegrated chloroplasts, and embedded in it the fungus bodies are distinctly found, assuming an appearance of hyaline vacuoles.

Special attempt has not been made to examine the infection of epidermis. Incidentally the growing fungus bodies were found in the epidermal cells near the infected wound of some plants (*Fagopyrum, Cucumis*, etc.). Probably, as may be inferred from the results of the experiments on the Leguminosae, young epidermis of the non-leguminous plants whose internal cells are susceptible may also be infected. To obtain a ready infection we *need inoculation on young thin-walled epidermal cells.* However, on account of their being small in size and of the difficulty of obtaining a clear view of the cell cavity an accurate result of the experiment is not expected without the aid of a microtome.

The results of the foregoing experiments with non-leguminous plants show that most of them are susceptible to infection, while some, however, are not able to nourish the invader. In the relation to the parasite the leguminous and non-leguminous plants reveal no distinction. To decide whether or not the susceptibility or resistance is exhibited in connection with the specific, generic, or family character and to know how wide does the range of the susceptible plants extend we must await future extensive investigations. Presumably it appears that the host plant would amount to an enormous number, if the examination is extended further.

IV. Chemotaxis in Relation to Infection.

1. Plant Tissues.

A. Living tissue.

Whenever the gametangia are brought to germination in a hanging

drop of water the released swarmcells are at first dispersed freely in the medium. After a while they approach the host tissue, upon which the germinating gametangia are borne, and their distribution in water is becoming denser around it. Finally they come close to the surface of the tissue exhibiting a creeping movement[1] until they become sedentary and encyst

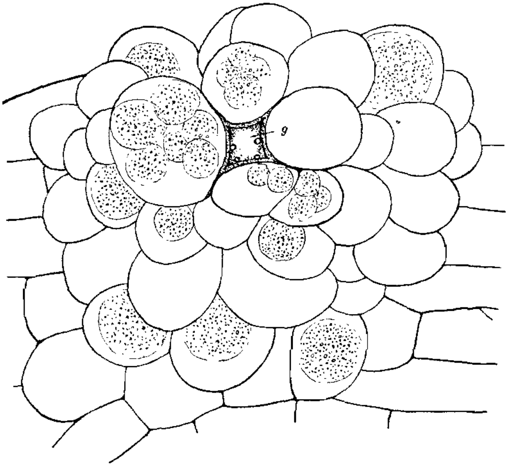

Fig. 9. A tumour caused by *Olpidium Viciae* on the stem of *Vicia unijuga*, showing its cells heavily infected by the swarmcells liberated from a gametangium (*g*). ×300

themselves. It may be noted that the encystment of the swarmcells may take place upon the surface of the cover glass and on the surface of the water drop, but nowhere are they found so densely as upon the surface of the host tissue. It gives us the idea that the swarmcells are chemotactically attracted by the host tissue. This idea is strengthened when the be-

1) In this and following places the "creeping movement" is used to express the swimming movement close to the surface of a substratum.

haviour of the swarmcells towards the tumour cells is observed. These cells, being developed around a host cell, are much swollen and are thin-walled, *assuming a young state. The swarmcells are gathered upon their* surface in great numbers, while those creeping on the surface of the neighbouring normal epidermal cells are remarkably few. In the field the infection of the tumour cells is ascertained to be always very pronounced (Fig. 9). This fact shows that the attraction by these cells is stronger than by the neighbouring epidermal ones which have advanced in development so far as to be provided with a thick outer wall. It suggests that there is a correlation between the infection of the cell and the diffusion from it of attractive substances.

This matter concerns the proper host plant of either *O. Viciae* or *O. Trifolii.* Yet as may be easily conceived, a similar relation would exist between the fungus and other plants which have already been ascertained to be infected.

To test this the experiment was conducted as follows. Introducing pieces of affected portions of the host in water of a vessel the suspension of the swarmcells was obtained in 20–30 minutes. In a hanging drop of this water a small piece of tissue of the examining plant was introduced. We observed that the swarmcells soon approached the piece and proceeded *to creep over its surface, the creeping ones increasing progressively in number.* Most of the plants listed in Table 1 gave a similar result, so that it is scarcely doubted that the successful infection of these plants, as already reported, is effected by virtue of the chemotactic response of the swarmcells.

Attention is now called to the species of the Leguminosae whose tissue has been unsuccessful of infection (Table 1). The question set forth is whether they are lacking in the attractive stimulant or not. To answer this question the following plants were examined:

Cassia mimosoides var. *nomame.*
Caesalpinia japonica.
Gleditschia horrida.
Albizzia Julibrissin.
Cassia occidentalis.

On *Cassia mimosoides* var. *nomame* approach of the swarmcells is not observed. On the contrary they are less in number in its neighbourhood than in other places of the medium. It shows that the tissue is rather repulsive.

On *Caesalpinia* the approached swarmcells soon retreat back. Later, some *individuals are creeping over the surface of a certain* cell row. As

a whole, the tissue seems to give off both attractive and repulsive substances.

On *Gleditschia* the forward and backward movement of the swarmcells are observed at a local spot of the piece. It appears to show that certain cells exert both attractive and repellent action. Sometimes a diffuse gathering of the swarmcells is observed on another place of the piece. We see that the attraction, if any, is not typical and is feeble.

On *Albizzia* the attraction is ascertained and the creeping movement of the swarmcells is obtained but it is not so remarkable as observed on the piece of the proper host. Perhaps it may be due to a less amount of an attractive substance to be diffused from the cell.

On *Cassia occidentalis* the attraction and the creeping movement can be observed as on the susceptible plants and the encysted swarmcells are found densely upon its surface.

From these experiments it may be stated that the swarmcells are not able to invade the living cell of *Cassia mimosoides* var. *nomame*, *Caesalpinia*, and *Gleditschia*, but there is a possibility that *Cassia occidentalis* and *Albizzia* may be infected. Therefore, the failure of infection of the latter plants, as mentioned in the previous experiments (Table 1), does not yet prove that they are in fact non-susceptible.

Similar experiments were carried out with the plants from other families, which have been used for the infection experiments (Table 2). As may be expected, the tissue of all susceptible species attracts the swarmcells equally well. Among the plants, the infection of which has appeared to be unsuccessful, *Oenothera biennis*, *O. Hookeri*, *Dahlia*, and *Lactuca* show a distinct attraction, leading the swarmcells to the creeping movement and subsequent encystment upon the surface. Hence it is probable that the cysts upon their surface may establish the infection. *Chrysanthemum* gives a similar but more prominent effect upon the swarmcells. Although the germinating cysts are able to perforate the cell wall, the fungus plasm migrated through it into the cell is prevented to attain the host plasm in a way as already given (Fig. 6). *Lycopersicum*, *Calystegia*, and *Rubus* relate to the fungus otherwise; approached swarmcells are deposited and round up to disorganize finally, or they are absent near the tissue as the result of its repulsion. We conclude with decision that these plants are absolutely resistant to infection.

B. Dead tissue.

The experiments have been undertaken with a view to know whether there is any difference in the chemotactic action between the living and

the dead tissue or not. It is evident that the attraction of the tissue is connected with the diffusion of some components of the cell content through the cell wall. The amount of the diffusing substances or the rate of their diffusion may be different in the living and the dead cells, or may vary according to the intervals of time, during which the tissues are previously laid in water. As *Vicia Faba* exerts very remarkable attraction, the experiments were made with its tissue.

Slices of nearly similar sizes were taken from a young stem, some from the fresh living material and the other from that killed with boiling water. One part of both living and dead slices was used after being washed with water for one or two minutes, while the other was used after being laid in water half an hour. For the sake of mere convenince we distinguish them as new and old slices. The reactions of the swarmcells, viz. their gathering to, creeping over, and encysting upon these slices, present a remarkable variation as mentioned below (Table 4).

TABLE 4.

Showing the reaction of the swarmcells to the tissue of *Vicia Faba* and its effect. 0, negligible; +, ++, and +++, feeble, moderate, and strong respectively.

Tissue	Attraction	Gathering	Creeping	Encystment
New living	Immediate & strong	+++	+++	+++
Old living	Gradual	+++	+++	+++
New dead	Weak	++	++	+
Old dead	Feeble	First +, later 0	First +, later 0	0

From this experiment we learn that the action of both new and old living tissues upon the swarmcells gives rise to a similar final effect, though different at first. Towards the dead tissue, especially the old one, the reaction of the swarmcells is weaker than towards the living one and finally it is no more perceived.

Instead of the material killed with boiling water, a steamed one was prepared and the slices from it were compared regarding the action upon the swarmcells with those taken from the living material. When the steamed tissue, after lying for a while in water, is introduced together with the living tissue in the medium of the swarmcells, we observe that the attraction is exhibited more strongly than is that of living tissue.

Remarkably, the swarmcells swim about densely around the tissue but the creeping and encysting ones are comparatively less in number. Later, the gathered individuals are dispersed and also the creeping ones are decreased in number, leaving a few encysted ones behind. In great contrast with it the creeping individuals on the living tissue are increased progressively in number during an interval of two hours and at length they are deposited on the surface of tissue in dense rows to encyst themselves. When the steamed tissue laid in water for twenty-four hours is used, the attraction, though feeble, is recognized during the first five to ten minutes. Later, the swarmcells do not show any reaction to it, and the creeping and encysting individuals are as few as upon the cover glass.

From the experiments given above it will be seen that both the living and the dead tissues exert the attractive action. Evidently they give off the stimulant into the surrounding medium. Judged from the behaviour of the swarmcells, the dead tissue seems to be exhausted of the stimulant sooner or later. It is no more doubted that the creeping movement of the swarmcells on the surface of the tissue is exhibited in connection with the diffusion of the stimulant. Remarkably enough, this movement is pronounced on the living tissue. The number of creeping individuals is increased minute after minute, while the condition is the reverse on the dead tissue. I may explain this feature of the creeping movement by assuming a constant supply of the stimulant by the living protoplast, which is enabled by its incessant metabolic action.

The creeping movement is further significant as indicating chemotaxis. The individuals at smooth swimming movement take an attitude of alarm or excitement on approaching the surface of the tissue. Without being stimulated such a behaviour would not be exhibited. From this manner of movement we may learn that the concentration of the stimulating substance is kept higher on the surface of the cell than in any other place of the medium, on account of which the swarmcells once arrived there do not leave the surface.

2. Plant Juices.

The attractive action of the plant tissue upon the swarmcells is evidently due to the presence of diffusible substances in the cells. We now proceed to examine whether or not the same substances exist in the plant juices. In this case the chemotactic action can be observed more clearly, as the capillary method is applicable. After filling a capillary tube with

the juice exudated from the cut surface of the desired turgescent plant[1], one end of the tube is sealed with wax or vaseline, and the other open end is introduced into a drop of water, in which the swarmcells are suspended. If the stimulant is contained in the juice in a sufficient amount, the swarmcells come immediately to the front of the mouth in great numbers, all in active bustling movement. Among the leguminous plants already used for the inoculation experiments (Table 1) the following species are chosen for examination :

Albizzia Julibrissin.
Caesalpinia japonica.
Cassia mimosoides var. *nomame.*
C. occidentalis.
Gleditschia horrida.
Lotus corniculatus var. *japonicus.*
Pueraria triloba.
Trifolium repens.
Vicia Faba.
V. unijuga.

Non-leguminous plants tested, in addition to those given in Table 2, are as follows :

Amarantus mangostanus L. (Amarantaceae).
Arisaema Thunbergii Bl. (Araceae).
Chelidonium majus L. (Papaveraceae).
Dioscorea Tokoro Mak. (Dioscoreaceae).
Erigeron canadensis L. (Compositae).
Geranium nepalense Sweet (Geraniaceae).
Glechoma hederacea L. (Labiatae).
Houttuynia cordata Thunb. (Saururaceae).
Hyacinthus orientalis L. (Liliaceae).
Lamium amplexicaule L. (Labiatae).
Lysimachia clethroides Duby (Primulaceae).
Melia Azedarach L. var. *japonica* Mak. (Meliaceae).
Morus bombycis Koiz, (Moraceae).
Oenothera Lamarckiana Ser. (Oenotheraceae).
O. muricata L. („).
O. odorata Jacq. („).

1) The juice is obtained, when necessary, by pressing the tissue.

O. sinuata L. („).
O. tetraptera Cav. („).
Paulownia tomentosa Kanitz. (Scrophulariaceae).
Phryma leptostachya L. (Verbenaceae).
Phytolacca esculenta Houtt. (Phytolaccaceae).
Pirus Malus L. (fruit) (Rosaceae).
Sanguisorba officinalis L. („).
Sedum japonicum Sieb. (Crassulaceae).
Silene Armeria L. (Caryophyllaceae).
Viola Patrini DC. (Violaceae).
Zingiber Mioga Roscoe (Zingiberaceae).

In most of these seventy-eight plants the juice induces the positive chemotaxis, the attractive power varying according to the different plants. Above all *Vicia Faba* furnishes an excellent juice for attraction. As a noteworthy fact the juice of *Cassia mimosoides* var. *nomame* is strongly attractive, though the tissue is rather repulsive. Perhaps the diffusion of the attractive substance from the tissue is feeble, while it abounds in the cell sap.

Of the remaining plants the following ones are worthy of special mention, as the swarmcells behave to their juices in unusual manners.

(a) *Solanum tuberosum* (infection is obtained)[1]. We have observed in the preceding experiment that the tissue attracts strongly the swarmcells and they encyst densely upon the surface of the tissue. Also the juice shows a similar strength of attraction, but the attracted swarmcells become sluggish soon and settle down. It is probable that the juice contains some substance detrimental to the swarmcells.

(b) *Lycopersicum esculentum* (infection fails). Swarmcells approached to the tube mouth are soon killed. The occurrence of a poisonous substance in the juice is very probable.

(c) *Calystegia japonica* (infection fails). Swarmcells are attracted to a certain distance from the tube mouth. When the juice is diluted with water, they approach nearer the mouth but soon fly away. We see that the juice contains both attractive and repulsive substances.

(d) *Dahlia variabilis* (infection fails). Swarmcells approached to the tube mouth and around it sooner or later become immovable and are deposited in succession. It appears probable that an injurious substance occurs in the juice.

1) In the bracket the result of the previous experiments is given.

(e) *Rubus trifidus* (infection fails). Swarmcells are densely accumulated at the tube mouth, stopping but moving the body.

(f) *Caesalpinia japonica* (infection fails). The behaviour of the swarmcells is the same as observed on *Rubus*. It may be noticed that the tissue is nearly inactive for chemotaxis and the juice is strongly acid.

(g) *Hosta Sieboldiana* (infection fails.) Swarmcells approaching the tube mouth stop on attaining a certain distance from it and are deposited there. It appears that the juice is attractive but injurious to the swarmcells.

(h) *Albizzia Julibrissin* (infection fails). The approaching swarmcells are killed. When the diluted juice is used, they come nearer to the mouth but are also soon killed. Its tissue has been ascertained to show a feeble attraction. Evidently the juice contains both attractive and injurious substances.

(i) *Gleditschia horrida* (infection fails). When approached to the tube mouth the swarmcells show a tendency to come to rest. Later, actively swimming individuals are found crowded round the mouth. As may be seen from the experiment with the tissue, the juice seems to contain a certain substance giving interference with the locomotory movement of the swarmcells.

(j) *Cucumis sativus* (the fungus establishes the complete development). Swarmcells form round the tube mouth a repulsion sphere becoming distinct with an increasing number of depositing individuals. When the juice is diluted, the swarmcells soon assemble densely round the tube mouth, maintaining an active movement.

(k) *Pueraria triloba* (infection is obtained). Swarmcells are attracted apparently, but their deposition soon takes place. When the juice is diluted, some come to rest before approaching the mouth, while some turn away. Here we perceive both attraction and repulsion.

(l) *Oxalis violacea* (the fungus establishes the complete development). Immediate approach to the tube mouth is not observed, but when the swarmcells attain a certain diffusion zone, positive chemotaxis is exhibited clearly. In the diluted juice the attraction is not apparent. Since the tissue gives off the attractive substance as evidenced by its infection, we assume that the action of the attractive component of the juice is disturbed by the co-existing repulsive or injurious component. It may be remarked that the juice is strongly acid.

(m) *Rumex japonicus* (the fungus establishes the complete development). Swarmcells attracted to the tube mouth are deposited after a while. It is

supposed that a high acidity of the juice exerts a certain influence upon the behaviour of the swarmcells.

(u) *Chelidonium majus.* The yellow juice is strongly poisonous and the swarmcells do not approach the tube mouth, as they are killed as soon as they attain a certain diffusion zone.

Thus in all plants just mentioned the positive chemotaxis is not presented typically. This is undoubtedly due to the action of certain components of the juice upon the locomotory movement of the attracted swarmcells. The nature of such components is not studied, but the characteristic behaviour of the swarmcells towards each plant and the divergence of the systematic relationship shown by these plants render it probable that such active components may be of widely different natures.

From the results of the above experiments we come to the conclusion that a certain attractive substance for the swarmcells occurs in almost all plants examined and that it is contained in the expressed juice and is diffusible to the surface of the cell.

It is a matter of fact that the plant tissue and its expressed juice would not relate similarly to the swarmcells. While in the tissue the substances to be diffused through the cell wall are essential, in the juice several organic and inorganic substances are present, originating from the cells of different natures, and they co-operate upon the swarmcells. For instance, the juice of *Pueraria* contains tannin which is originated from special cells. In a study of *Synchytrium minutum* parasitic on some internal cells of *Pueraria* it was ascertained that the expressed juice is strongly repulsive to the swarmcells, while tannin was also found to be repulsive. The injurious or repellent action of the juice from the same plant upon *Olpidium* which, as shown in Table 1, is able to develop in some of its living cells would be due to the presence of tannin.

In the *Chelidonium*-juice the orange-yellow sap in the laticiferous vessel is mixed in abundance and may be essential in killing the swarmcells. Perhaps the sap of the usual parenchymatous cells would contain the attractive substance but in the juice its action is not manifested on account of the presence of the poisonous substance. The contents of the laticiferous vessel in *Taraxacum* and *Lactuca* and of the latex cell in *Morus* show no decisive injurious action or at least are not effective for disturbing the attractive action of another component of the juice.

So far we have ascertained that the juices from most of the examined plants induce the typical positive chemotaxis. From this fact we emphasize that innumerable, water-soluble substances as assumed to be found in so

many kinds of plants as already mentioned do not affect the locomotory movement of the swarmcells.

3. Chemical Substances.

The chemotactic action of plant tissues and juices having been ascertained, our interest is now centered upon the problem of the chemical nature of the stimulant contained in the plants. At present, however, I am not able to treat this subject thoroughly and in the following only a brief account will be given in a preliminary form.

The concentration of chemicals tested was not determined accurately. At first their solutions at a moderate concentration were prepared and after testing their action stronger or weaker solutions were used when necessary. This method, very rough as it may be, was practically sufficient to determine whether the examined chemicals are attractive or not.

Regarding the knowledge of the chemotactic sensibility in the swarmcells of fungi attacking the living plants we owe much to a very elaborate work of Müller (1911) on the Chytridiaceae and to a recent study of Arens (1929) on the Peronosporaceae. According to the former author *Rhizophidium* and *Olpidiopsis* (*Pseudolpidium*) are attracted by several protein substances, while the latter author mentioned P- and OH-ions as causing the positive chemotaxis upon *Plasmopara viticola*. Bearing in mind that *Olpidium* is allied to *Rhizophidium* and *Olpidiopsis*, and supposing that all these fungi might perhaps be similar with respect to the chemotactic response, I proceeded as first to test some available protein substances and their derivatives.

Haemoglobin, casein, Witte-pepton, and diastase, which are ascertained *in Müller's fungi to be more or less attractive, are chemotactically* inactive to *Olpidium*. Legumin and pepsin appear to exhibit a weak attraction but there is some doubt about the purity of these chemicals.

Asparagin exerts no chemotactic action.

It may be noticed that Teruuchi-pepton and flesh extract are found to be the excellent stimulants. At their definite concentrations the swarmcells rush to the mouth of the capillary tube and enter the tube in great numbers. Flesh extract is of course a mixture of several organic and inorganic compounds, so that the attractive component is unknown. The attraction of Teruuchi-pepton might certainly be due to the occurrence of certain substances other than pure pepton.

A few carbohydrates, such as saccharose, glucose, laevulose, and lactose, are examined but none of them gives a positive result of chemotaxis.

Some acids, hydrochloric, acetic, tartaric, malic, and citric, are tested at various concentrations. When their weak solutions are used, the swarmcells pass freely the front of the tube mouth without presenting any sign of stimulation. When the concentration is increased, they are deposited near the mouth or form a wider or narrower vacant space around it. To a solution of hydrochloric acid indicating a pH of 3.0 they retain the swimming activity near the mouth longer than to a more concentrated solution, but none of them enters the tube. As far as the present study extends, the positive chemotaxis is not induced by the acids. On the contrary H-ion is repulsive to the swarmcells.

Next the following salts are examined:

Potassium chloride
Potassium bromide
Potassium iodide
Potassium chlorate
Potassium nitrate
Potassium bisulphate
Potassium sulphate
Potassium acetate
Potassium citrate
Potassium malate
Potassium oxalate
Dipotassium phosphate
Sodium chloride
Sodium chlorate
Sodium nitrate
Sodium sulphate
Sodium acetate
Sodium citrate
Sodium malate
Sodium oxalate
Disodium phosphate
Ammonium nitrate
Ammonium sulphate
Ammonium tartarate
Primary calcium phosphate
Magnesium nitrate
Magnesium sulphate

Of these only potassium salts are attractive, while all others are entirely indifferent. The potassium and sodium salts exhibit a great contrast in their action upon the swarmcells. This is clearly demonstrated when two tubes, one containing the potassium and the other the sodium salt, for instance KCl and NaCl respectively, are inserted almost simultaneously into the same medium of the swarmcells. We conclude that the swarmcells are attracted by K-ion, while Na-, Ca-, Mg-, Cl-, ClO_3-, NO_3-, SO_4-ions, etc. are entirely indifferent towards chemotaxis or at least are not attractive.

Of hydroxides only KOH and NaOH are examined. At a moderate concentration KOH attracts the swarmcells immediately and densely to the tube mouth, but in the course of the swimming movement their body tends to deform into an elongated and slender form, before becoming immovable

ıd disorganized. In a more dilute solution showing the pH value of 8.0 ıe swarmcells immediately crowded to the tube mouth are able to swim ɔout still longer with their bodies underformed. In the whole aspect of :traction this solution is comparable with the juice of *Vicia Faba,* which most prominent in attraction among the juices examined. It may be ɔted that the *Vicia*-juice is a little acid (pH 6.0-6.2).

NaOH does not at any concentration induce an assemblage of the varmcells. Freely swimming individuals take an attitude of alarm on taining a certain zone of diffusion and immediately turn away from the ntre of diffusion. This feature indicates the repellent action of NaOH. s may be judged from the inactivity of the sodium salts given above, we ınclude that OH-ion is a repulsive component.

It is clear that in a solution of KOH the attraction of K-ion over- ›wers the repulsion of the co-existing OH-ion. Obviously the deformation ' the attracted swarmcells and their final disorganization are ascribed to ı injurious action of OH-ion. From this fact we deduce that the swarm- lls are chemotactically less sensitive to OH-ion than to K-ion.

From the foregoing we draw the conclusion that the swarmcells of *lpidium Viciae* and *O. Trifolii* are strongly attracted by K-ion and are ·gatively chemotactic to H- and OH-ions. To chemicals giving other ions ıd to several organic compounds examined they are chemotactically insen- :ive.

4. Conclusion.

We have so far ascertained that the swarmcells of *Olpidium* are attracted ɣ the tissue and juice of most of the examined plants. The attraction of e tissue is evidently due to the presence of a chemotactic substance which ısses from cell to cell through the wall. Also the same substance is con- lered to exist in the expressed juice which represents essentially the cell p. An almost universal occurrence of this substance in higher plants is arcely doubted. Determination of its nature is not attempted on analysing e diffused substances or the expressed juices. However, on examining a ımber of chemical substances only potassium compounds are proved to be tractive. When we take into consideration that potassium is of universal currence in living cells (cytoplasm and vacuole) of higher plants as re- ›rted by Weevers (1911) and recently by Penston (1931), the attraction ' the plant tissue and juice may reasonably be due to the existence of ›tassium in the ionized form, no matter whatever some additional attractive bstances may occur.

On comparing the living and the killed tissues for the attracting action we have pointed out that the action is constant and more durable in the living tissue but is often stronger at an early and weaker at a later period in the killed one. As the stimulant is ascertained to be potassium, this feature of attraction is easily explicable. When the cell is killed, potassium compounds contained in cytoplasm and vacuoles are rapidly diffused and their resource is soon exhausted, while their diffusion from the living cell may be a result of the physiological activity of the living cytoplasm and may take place incessantly as long as the cytoplasm is alive.

It is more than evident that there are several other substances, organic and inorganic, which are diffusing from the cell or contained in the cell sap. Whatever their nature may be, it is very likely that in a greater majority of plants so far examined they are chemotactically inactive to the swarm-cells. However, it should not be overlooked that some plants contain certain substances which may be of such a nature as to give a repellent or injurious action upon the swarmcells. Whether they are specific or not to certain varieties, species, genera, etc. is at present not decided, but it is evident that the diffusion of such substances in a sufficient amount prevents the approach of the swarmcells to the cell for invasion.

As one of the chief results of the chemotactic experiments we may mention the relation between hydrion concentration in plant cells and their infection by the fungus. The reaction of the juices, as far as the test by litmus paper is concerned, is in almost all plants distinctly acid, being in some weaker or stronger than in most plants. For instance, *Vicia Faba* shows a low acidity, while *Oxalis violacea* is strongly acid. As previously mentioned, the wound surface of both plants is ready to infection and it is even possible to develop the fungus upon them. While the juice of *Vicia Faba* immediately attracts the swarmcells to the mouth of a capillary tube, that of *Oxalis violacea* exerts a strong repellent action. Thus the chemotactic action of the juice upon the swarmcells is an opposing one in both plants, though their intact cells accord in attracting the swarmcells to infection. With a view to knowing how this contradiction is brought about the pH value in the expressed juice of both plants was examined and it was found to be 6.0–6.2 in *Vicia* and approximately 2.8 in *Oxalis*. We have already mentioned that a solution of HCl indicating a pH of 3.0 causes the negative chemotaxis. Hence it is certain that the repulsion of the *Oxalis*-juice is assignable to a high acidity. We can state decidedly that the acidity of the cell sap exerts but little influence upon the infection of the cell by *Olpidium*.

In phytopathology the relation between hydrion concentration in plants and the resistance to the parasitic diseases has received considerable attention and elaborate works have recently been done by many authors. As may be seen from the review by FISCHER-GAUMANN (1929, p. 194), an exhibition of resistance in connection with hydrion concentration remains doubtful. As far as the fungi developing in the living cell are concerned, it would be rather surprising that the acidity of the cell sap interferes in any way with their invasion. Entracellular parasites, such as *Olpidium*, *Synchytrium*, and *Plasmodiophora*, or the haustorial parasites, such as the Erysipheae, Uredineae, and Peronosporaceae, take their food directly from the living protoplast. Therefore, the research into the relation between the nature of the cell sap and the development of the parasitic fungi included in the group just mentioned would be quite meaningless. It must be borne in mind that MÜNCH'S perthophytes which establish saprophytism on living plants are irrelevant to the question. The source of their nutrition is the disorganized cell content. The juice of the dying cell is no more comparable with that of the living one and evidently it is misleading to lay much stress upon the degree of hydrion concentration in the expressed juice from the living cells as indicating its real concentration acting upon such fungi.

V. Relation of the Host Plasm to Infection.

Regarding the process of infection exhibited by the swarmcells subsequent to encystment upon the host cell an account has already been given in an earlier paper (KUSANO, 1912). Under favourable conditions the cyst germinates in an hour or two on producing a short infection tube which perforates the cell wall. As soon as the apex of the tube attains the plasmic layer beneath, the content of the cyst migrates through the tube into the host cell and is suspended in its plasm as a naked spherical mass (Fig. 10, *A*). With this process the infection is said to be accomplished. The cyst occurring on the cover glass or on the free surface of water, as is often observed, sends out a long fine filament instead of a short infection tube, remaining blind forever. As to how this divergent mode of germination is brought about we have no evidence to explain. However, it may be mentioned that the following fact obtained in the present investigation appears to throw a certain light upon the matter. When two pieces of tissue of *Vicia Faba*, the one being living and the other being killed with steam, were introduced in a suspension of the swarmcells (*O. Viciae*),

numerous cysts were afterwards found over the surfaces of both pieces. On the next day the cysts on the living tissue were already emptied by a large percentage, sending their contents into the host plasm (Fig. 10, *A*). In the meantime those on the dead tissue were also mostly germinated, but like those on the cover glass or floating on the surface of water, gave rise to the long filaments which, perforating the cell wall, made their way into the cell cavity (Fig. 10, *B*). The filament is filled with the plasm, remains blind, and finally is disorganized.

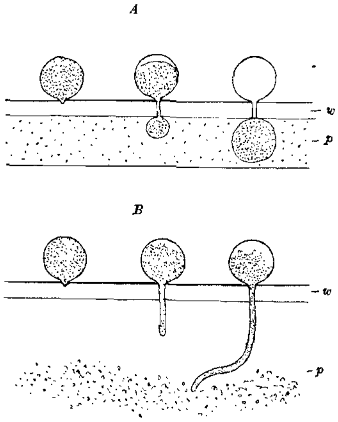

Fig. 10. Germination of cysts on a living (*A*) and killed cell (*B*). *w*, cell wall; *p*, host plasm. Diagrammatic.

From the above we see that the attractive power is still maintained in both tissues, while the cysts are going to germinate. In great probability the direction of the infection tube and the filament is determined by K-ion which is effective for attracting the swarmcells. This theory may be supported by the fact that the filament does not enter the cell of the dead tissue which, being previously laid in water for a certain length of time, seems to decrease considerably the amount of the stimulant.

For the germinated cysts the condition of the cell cavity is very divergent in both tissues. In the living tissue the cell wall is underlined with the plasmic membrane, while in the killed one plasmolysis or disintegration of the plasm leaves a clear space under the wall for some extent. The fungus is able to reach the plasm of the living cell by sending out a very short infection tube, the length of which just corresponds to the thickness of the cell wall. The formation of a long filament entering the dead cell tends to show that the fungus is as if attempting in vain to attain the living plasm. We come to the view that the chemical attraction of the living cell is a means to bring the fungus in close contact with the host plasm. The further process of infection, that is, the opening of the infection tube and the immediate release of the fungus plasm through the opening, may be attributed to a certain interaction between the living plasms of both the fungus and the host cell. As to the mechanism operative in this case we know but little. However, I may call attention to the fact that the cytoplasm of higher plants in general contains potassium (WEEVERS, 1911; PENSTON, 1931). If we assume that the fungus plasm in the cyst retains the character to be responsive to K-ion, its migration into the host cell may be caused by the chemotactic attraction of the living cytoplasm.

From the facts given above we may state that the infection process involves three phases, viz.

(a) Approach of the swarmcells to the host cell by chemotaxis and the subsequent encystment upon the surface of the cell.

(b) Autonomic germination of the cyst, giving rise to the infection tube which, being responsive to a chemotropic action of the host cell, makes its way into the cell cavity on perforating the cell wall.

(c) Migration of the fungus plasm through the opening of the tube into the host plasm.

It will be seen that the first two phases may be exhibited independent of the living cell, while the last phase is induced by the action of the living plasm. It is interesting to note that this action is exerted even by the plasm which is uncongenial to the invading fungus, as already demonstrated, for instance, in the potato (Fig. 8).

VI. Specific Characters of Olpidium.

Both forms of *Olpidium* exhibit no remarkable morphological diversity, and in a former paper (KUSANO, 1929 a) I mentioned the size of the gamete as the sole specific character deserving to be a distinction between two

fungi. In the present investigation a further observation reveals the fact that *the structure of cytoplasm in the gamete differs slightly but distinctly* from each other in both fungi. The gamte of *O. Viciae*, which is, as reported before, larger than that of *O. Trifolii*, contains the plasmic granules a little coarser than those in the gamete of *O. Trifolii*.

It might perhaps be questioned whether these characters of the gamete may vary or not, if the fungi develop on different hosts. Decision of this point is very important in determining both fungi as identical with or different from each other. Hence an attempt has been made to compare the given characters of the gametes of the same fungus developed on different *hosts and of different fungi developed on a common host*. It gave the following results:

(1) Each of *O. Viciae* and *O. Trifolii* developed on *Pisum sativum* gives the gametes which retain the character of those developed on the proper host.

(2) *O. Trifolii* developed on *Vicia unijuga* produces the gametes easily distinguished from those of *O. Viciae* on the same plant.

(3) The gametes of *O. Viciae* produced on *Vicia Faba* for three successive generations remain constant in their morphological character, not revealing any differences from those produced on the proper host, *Vicia unijuga*.

Thus we convince ourselves that the morphological characters of the *gamete merit diagnostic value in both species of Olpidium*.

The infection experiments in the present investigation teach us that the two fungi can develop on the same plant, showing the same range of hosts. However, their action upon the same host is not always similar; while some hosts affected with either *O. Viciae* or *O. Trifolii* appear healthy, others produce the typical tumour by *O. Viciae* but not by *O. Trifolii*, and in still others the relation to both fungi is reversed in this respect, as shown in the following table (Table 5).

TABLE 5.

Showing different reactions of hosts to *Olpidium Viciae* and *O. Trifolii*. Formation of the tumour is indicated by +.

(Host)	(*O. Viciae*)	(*O. Trifolii*)
Vicia Faba.	+	—
V. sativa var. *angustifolia*.	+ ?	—
V. unijuga.	+	—
Pisum sativum.	+	—
Trifolium repens.	—	+

TABLE 5. (Continued)

(Host)	(O. Viciae)	(O. Trifolii)
T. pratense.	—	+
Astragalus sinicus.	—	—
Glycine Soja	—	—
Phaseolus vulgaris.	—	—
P. angularis.	—	—

As far as the experiments extend, *O. Viciae* causes the tumour only on *Vicia* and *Pisum*, while *O. Trifolii* gives a similar effect upon *Trifolium* only. It is further noted that *O. Trifolii* developing on the epidermis and the wound surface of *Vicia unijuga* causes on the invaded cell a discolouration often earlier than usual and similarly *O. Viciae* on *Trifolium repens.* We conclude that the pathogenic action of both fungi upon certain plants is not the same[1]).

Very slight morphological difference exhibited between *O. Viciae* and *O. Trifolii* makes us recall the morphological-biological forms of a species. As pointed out by FISCHER-GÄUMANN (p. 153), it may be a matter of convenience whether we take them as sub-species under a species or retain them as two distinct species. Although the herbarium specimens do not reveal any diagnostic distinction between the two, indicating them to be the same species, yet from the pathological point of view we would rather prefer to treat them as distinctive from each other.

Olpidium Brassicae (Wor.) Dang., the best known species of the genus, is reported by BENSAUDE (1928) to occur on the root of tomato and tobacco, divergent in relationship from cabbage, upon which the fungus is originally known. The structure of the resting cell differs remarkably from that of either *O. Viciae* or *O. Trifolii.* Our fungi can develop on cabbage but not on tomato. They are injured by certain chemical substances contained in the living cell of tomato. In this respect they can also be distinguished from BENSAUDE's *Olpidium*.

Our fungi are positively chemotactic to the tissue and juice of *Taraxacum* and invade its living cell. However, they are not able to develop in the invaded cell as they are disintegrated soon after invasion. On this account identification of our fungi with *O. simulans* (DeBary et Wor.) occurring on *Taraxacum* is not admitted.

It is scarcely doubted that chemotaxis is also concerned with the

1) In an investigation not yet published it has been ascertained that both fungi differ from each other in respect to the mode of sex differentiation.

infection by *O. Brassicae* and *O. simulans*, though we know but little about the kind of the stimulant. Irrespective of whether K-ion is active in this respect or not, it is almost certain that their relation to some of the chemical substances contained in their hosts or to the host plasm is different from that of our fungi to the same substances or to the protoplast of the same plant.

VII. Discussion of Results.

The present work, in which attempt has especially been made to know the parasitic nature of the two forms of *Olpidium*, has brought forth the facts which seem to have a more or less bearing upon the principle underlying the parasitic diseases. When viewed from different standpoints several subjects are proposed for discussion. In the following some of these will be dealt with.

In treating of the host-parasite relationship attention should be paid to the distinction between the parasite and perthophyte as attempted by MÜNCH (1929). Since in these types of the disease-producing fungi the mode of nutrition is radically different, we are impressed with a great difficulty to discuss several problems involved in their relation to the host along the same principle. The fungi, with which we have worked out, are true parasites, so that in the account given below the reference to the works on perthophytes is almost omitted.

1. Range of Hosts.

As far as the field observation goes, *Olpidium Viciae* and *O. Trifolii* appear so much specialized as to be monophagous or at least as to limit their hosts respectively to *Vicia* and *Trifolium*. The inoculation experiments, however, have shown that several other genera of the Leguminosae are infected by both fungi. If the infection of the extra-epidermal cells is taken into consideration, the range of hosts is extended extraordinarily wider over many families of higher plants. From the general feature of the fungus development on these plants so far examined it may be predicted that a further examination would extend the range still wider. Since the susceptibility of the plant to these intracellular parasites is an expression of the susceptibility of its living cells, the results of inoculation experiments with the extra-epidermal cells would justify the statement that *Olpidium* under consideration is in fact plurivorous.

On arriving at so divergent a conclusion on the limitation of the hosts from that induced from the field observation it may be proposed to distinguish two types of the host according to the manner of reaction towards the parasite. In one type the typical symptom distinctly recognizable in external appearance is produced. Practically, as phytopathologists usually do, we take it as an indication of susceptibility of plants to the attack of any fungus. However, the host of the other type does not give any visible symptom, the affected portion appearing quite normal. On the macroscopic examination the presence of the developing fungi on such a host is easily overlooked.

An exactly similar instance is known in the case of virus diseases (cf. SMITH, 1930, p. 45; QUANJER, 1931, p. 585). The plant infected by a virus shows no sign of the disease, but when the virus is transmitted from it to the susceptible plant, the typical disease is produced. Such a disease-tolerant plant is designated as the carrier. This is true in our case and the swarmcells developed upon the carrier may be transmitted to the host of the first type. For example, *Glycine Soja*, *Phaseolus angularis*, *P. vulgaris*, *Vigna Catiang* var. *sinensis*, *Astragalus sinicus*, and others may be mentioned as the carriers of *O. Viciae* and they transmit the disease to *Vicia unijuga*, *V. Faba*, and *Pisum sativum*. Similarly these plants may carry *O. Trifolii* to *Trifolium repens* or other species of the same genus.

Little attention seems to have hitherto been called to the existence of the fungus carrier, though it may have a great significance upon the distribution of plant diseases. As far as known, the potato wart disease furnishes a likely instance. According to an extensive study of KÖHLER (1925, 1927) some highly resistant varieties of potato may be infected by *Synchytrium endobioticum* but present almost no visible symptom. In these varieties the complete development of the parasite is often disturbed (subinfection) but it is sometimes successful. Therefore they may carry the fungus in a healthy condition and propagate it in the field upon the susceptible varieties.

It may be noted that, though the fungus carrier is susceptible to the infection by the parasite, it is practically not diseased. FISCHER-GÄUMANN (p. 5–6), retaining the meaning of immunity to a narrow sense as prevailing in animal pathology, accept as one of the cases of true immunity, when an affected plant shows no response to the parasite. Following these authors it may be admitted to say that the fungus carrier is immune from the disease.

2. PARASITIC AFFINITY.

The swarmcells of *Olpidium* are chemotactically respousive to K-ion,

and, if any plant contains the potassium compounds diffusing to its surface, the fungus is attracted by that plant. We can say, therefore, that the fungus and the plant are brought into a close association by means of K-ion. This association is antecedent to infection. Without it both organisms are entirely indifferent to each other; the fungus does not attempt to invade or even to approach the plant. *We look upon this relation as the parasitic affinity.* Since potassium is of universal occurrence at least in higher plants, we consider the affinity group to be very large.

Of course it must be borne in mind that the degree of the chemotactic response of the fungus, and in consequence the intimacy of the affinity, is correlated with the amount of K-ion given off from the plant. If the amount is too small to cause the attraction, both organisms are not practically brought into a close association. However, in limiting the affinity group such a quantitative relation of K-ion is dismissed from consideration.

Other fungi which establish parasitism by the agency of the swarm-cells afford a similar instance of the parasitic affinity. *Rhizophidium pollinis* occurring on pollen grains of conifers is attracted by the genuine proteins which are universally found in plants. *Olpidiopsis Saprolegniae* on *Saprolegnia* is attracted by proteins and their nitrogenous derivatives, which are also commonly found in plants. According to chemotactic experiments carried on some years before, *Synchytrium minutum* on *Pueraria* appears to be attracted by several organic and inorganic acids and salts, some of which may occur in many plants. *Plasmopara viticola* on *Vitis* is attracted by P- and OH-ions, the former of which may be found in a greater number of plants. Although these fungi, like *Olpidium*, occur on particular kinds of plants, their affinity group may be said to comprise a vast number of plants.

On announcing such a conception of the affinity it may be questioned whether or not the stimulant, by means of which the swarmcells are brought to the surface of the cell, is effective for causing their entrance into it. It is true that the chemotactic behaviour of the swarmcells come to an end on undergoing encystment upon the surface of the plant or cell, and there is not in fact any evidence to prove that the sensibility towards the same stimulant is still retained in the encysted fungus bodies. Presentation of a great divergence in the process of invading the plant by these fungi makes the matter greatly complicated. In *Synchytrium* the encysted body is nothing else than a naked plasmic mass and it enters as a whole into the host cell. In *Olpidium* and *Olpidiopsis* the content of the

cyst migrates into the cell, leaving the wall of the cyst behind. In *Rhizophidium* the encysted body remains on the surface of the cell and sends out rhizoid-like mycelia into the cell. In *Plasmopara* it gives rise to a mycelium which advances through the stomata to the intercellular space and sends out haustoria into the neighbouring cells. In the last case of infection the formation of haustoria and the approach of the swarmcells apparently do not seem to be induced by the same cause. However, in *Synchytrium* the approach of the swarmcells to the surface of the cell and an immediate advance in an immotile form into the cell cavity do not seem to be induced by different causes. In the case of *Olpidium* the fact that the plants attractive to the swarmcells are mostly infected would support the view that K-ion would still be effective for leading the encysted fungus bodies into the host plasm, from which potassium is emanated. The non-infection of some plants which attract the swarmcells is not yet decisive as proving either the failure of any stimulant inducing the infection or the loss of the responsive character of the fungus to the existing stimulant. About this point we will consider later.

The parasitic affinity here proposed is an expression of a mutual relation between host and parasite. Considered from the part of each organism this relation may be expressed in other terms; the parasite is provided with a character of infectivity ("Aggressivität"), that is, an ability to invade certain plants and the host has a character of susceptibility, that is, a property to be invaded by certain parasites. In the present case the infectivity is a representation of the responsive character of the parasite to K-ion, while the susceptibility of the host is an indication of the presence of K-ion. Here the host-parasite relationship is initiated by the agency of K-ion. FISCHER-GÄUMANN (p. 8) states, "Da ferner die Aggressivität eines Parasiten verschiedenen Pflanzen gegenüber eine verschiedene sein kann, so führt sie zur Wirtwahl." In the conception mentioned above the infectivity of a parasite is genotypic and its variation towards different plants is inconceivable without consideration of the influence of external conditions upon the parasite. Hence the variable infectivity as expressed by the above authors is likely understood as a representation of variable degrees of susceptibility in different plants. In our case the degree of susceptibility may depend upon the diffusible amount of potassium. Moreover, some potassium-containing plants may be provided, as will be discussed later, with such characters as are effective for protecting them from the attack of the parasite. These characters would limit the range of hosts among the plants of the affinity group.

The conception of the parasitic affinity appears to correspond in essential points to that of KÖHLER (1929). While I confine myself to the group of fungi which show a true parasitic nature, excluding MÜNCH's (1929) perthophytes, he seems to embrace both parasites and perthophytes. He says (p. 226): "Der Affinitätskreis eines parastischen Pilzes umfasst diejenigen Ordnungen, Familien, Gattungen, Arten oder Varietäten, die den Pilz zu einer Aufschliessungsreaktion veranlassen. Als Aufschliessungsreaktion ist anzusehen, wenn der Pilz durch Eindringen oder durch Ausbildung von Saugorganen oder durch Abscheiden von Giften oder auf andere Weise versucht, den Zellinhalt des Wirtgewebes seiner Ernährung dienstbar zu machen." Regarding the parasitic relation of the Uredineae, the true parasite, to the host he conceives the presence of the "Bereitschaftsfaktor," explaining (p. 240): "Als Bereitschaftsfaktor wäre der von der Wirtzelle ausgehende Reiz anzusehen, der den Pilz zu einer Aufschliessungsreaktion, in diesem Fall also zur Absonderung gewisser spezifischer Stoffe veranlasst."

In the case of true parasites, to determine whether the secretion of a certain specific substance by the parasite is due to the stimulus exerted by the host or is independent of it, we need further consideration[1]). As to how the fungus is caused to penetrate into the living cell it appears likely that prime importance will be attached to the chemotactic or chemotropic stimulus exerted by the cell. First on responding to this stimulus the fungus would attempt to penetrate into the cell. Therefore, we assume that the parasitic affinity is initiated with chemotaxis or chemotropism. On this account KÖHLER's "Aufschliessungsreaktion" would be understood as the chemotactic or chemotropic reaction. The stimulant for this reaction marks the affinity group. If, for instance, a certain family, genus, or species represents the affinity group for a fungus, the stimulant should be of such a kind as to be specific to that family, genus, or species. On the other hand, if the plant is not provided with the stimulant, the fungus would be indifferent to it, and the affinity is not established between the fungus and the plant.

According to my conception the affinity group is assumed to be generally very large, as far as the fungi, the chemical stimulant for which is precisely determined, are concerned. As to whether or not it is in other fungi so small as to comprise only a single family, genus, or species

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1) Refer to the "Die Faktoren des Angriffes des Parasiten auf den Wirt" by FISCHER-GÄUMANN (1929, p. 272).

we are not yet decisive, although such a narrow range of hosts is familiarly known to us.

The indifference of the fungus to a plant is only significant as to the infection and does not express anything about the nutritive condition of its protoplast for the fungus. Whether the protoplast is able to nourish the fungus or not is to be decided after both come face to face. Theoretically it appears possible that among chemotactically indifferent plants there may occur those, in which the protoplast, like that of the host plant, affords a beneficial substratum for the development of the fungus. From the lack of the stimulant, however, the fungus is not able to detect such plants to gain entrance into their cells.

3. Nature of Resistance.

Resistance to the attack of the fungus is a problem to be treated in connection with the parasitic affinity. In the present case the group of the affinity is considerably large and the inoculation experiments have shown that both *Olpidium Viciae* and *O. Trifolii* are plurivorous. However, in the field they appear so much specialized as to be considered almost monophagous. We now proceed to consider how this difference between the natural and the artificial conditions is brought about with respect to the range of hosts and further what is the nature of resistance.

In the process, through which the parasitic development of fungi is accomplished, several phases may be marked. Relating to the fungi under consideration the following ones may be essential:

(a) Association of the fungus and the host.

(b) Approach of the swarmcells to the susceptible cell by chemotaxis and their subsequent encystment upon its surface.

(c) *Perforation of the cell wall by the germinating cyst, being followed* by the migration of its content into the host plasm.

(d) Congeniality of the host plasm with that of the residing fungus, whereby the latter is able to develop.

Disturbance of the process at any phase gives rise to unsuccess in the parasitic development of the fungus and results in what we broadly express as the resistance of the plant. In the following we will consider how and at what phase such distnrbance may occur.

(1) Habit, growth form, etc.

To establish parasitism the swarmcells are required to associate with the young portion of the plant. To meet this requirement the presence of

liquid water over that portion is of first importance. In connection with this the habit and the growth form of plants may be taken into consideration.

The proper host plants, *Vicia unijuga* and *Trifolium repens*, are perennial. Therefore, a stock once affected is liable to be attacked every year, since the soil around it is infested with the resting cells, by which the primary infection takes place. Several cultivated plants given as the artificial host are annual and, unless the soil is previously infested heavily with the resting cells, there is a less probability of the infection of their seedlings. In some cultivated plants the seedlings appear in seasons other than those, in which the resting cells are about to germinate. For instance, *Vicia Faba* and *Pisum sativum*, though they are readily susceptible to *O. Viciae*, are generally planted in autumn and in the next spring the seedlings attain a considerable size, so that the swarmcells liberated in the soil at this period are not able to attain the young portions.

The growth form of the plant is related to the attack of the fungi. As infection is obtained always on the presence of liquid water, it is obvious that the young portion is subject to affection when it lies beneath the other older portions, being protected against drought. *Trifolium repens*, being a creeping plant, affords a good example. Its stem grows creeping over the surface of the ground, carrying the upright leaves with long petioles. The gametangia on the affected leaves liberate the gametes on getting water, which, being carried down by water, attain the young portion of the stem. In the degree of susceptibility *T. pratense* does not differ from *T. repens*, as evidenced by the inoculation experiment. However, the young portion of the upright shoot lies higher and higher above the ground, being liable to be exposed to drought. Even though it is once attacked by the fungus, the gametes formed would not be able to attain the growing portion of the stem.

The stem of *Vicia unijuga* grows erect. A heavy infection by *O. Viciae* takes place in spring when the young shoots are appearing above the ground. The swarmcells released from the gametangia are essentially disseminated by rain water to the lower level, and generally the upper, lately grown portion of the host remains free from the attack of the fungus. On this account the fungus prospers only in the early part of the vegetative season, while the fungus on *Trifolium repens* can obtain the foothold throughout the growing period of the host, whenever the external conditions are favourable.

The character of the epidermal system is also an important factor for the infection. For instance, the young shoots of *Trifolium repens* and *Vicia*

unijuga are easily wet. In particular their surface in *Trifolium repens* becomes mucilaginous on wetting. The young shoots of *T. pratense* are densely tomentose and almost unwettable. In addition the bud is tightly inclosed *in the membranaceous stipule*. We need no explanation how these obstacles hinder the association of the swarmcells and the epidermal cells.

The above instances will be sufficient to explain that the habit, the growth form, and the epidermal system, each takes a part in keeping the plant from the fungus attack. In this case the unaffection of the plant is merely an indication of the disturbance in the association of the fungus and the living cell, with which the host relation is established. In a strict sense it does not express anything about the resistance of the cell itself, though on the part of the entire plant body the effect may appear to manifest the resistance in a broad sense.

(2) The cell wall.

It has already been shown that the infection of the internal cells takes place more readily than that of the epidermal cells and the infection of the young epidermis than that of the old one. These facts point out that the nature of the cell wall controls the infection.

In other fungi the study of many authors on the resistance offered by the cell wall attaches much weight to the question as to whether the penetration of the fungus in the wall is by a chemical or by a mechanical action. It is of course obvious that the wall may in some cases withstand such action, thus keeping the plant from the invasion of the fungus. However, before going into a consideration of the process of penetration I may call attention to the causal relation of the cell wall to the penetrating action of the fungus. In the case of *Olpidium* I am inclined to the view that *the hinderance of the wall to the diffusion of the attractive substance is* essential in preventing the infection. It is evident that the outer wall of the epidermis is less permeable to gases and water than the wall of the internal parenchymatous cells in the same-aged portion of the plant. If the wall is less permeable or impermeable to the attractive substance, the swarmcells occurring on its outer surface would be indifferent towards infection just as on the surface of any plant not included in the affinity gronp and do not attempt to perforate the wall. Therefore, in the true sense the withstanding action by the wall is in no way presented. It would be natural to conceive that the wall so constructed as to be easily permeable to the soluble substances may be at the same time easily penetrated by the fungus and *vice versa*. That the wall may offer more or less resistance to penetration is evident, but in the light of chemotaxis the view presented above would

have a greater weight for explaining the participation of the cell wall in the resistance of the cell to the fungus attack.

SALMON's xenoparasitism (FISCHER-GÄUMANN, 1929, p. 71) is interesting in this connection. He succeeded in the infection of barley by *Erysiphe graminis tritici*, when it is wounded or treated with narcotics. The mildew fungus, like *Olpidium*, is a true parasite and its parasitism is established by sending haustoria generally in the epidermal cells. Susceptibility in this case would be explicable when we assume an easier diffusion of a stimulating substance through the cell wall. In *Olpidium* I may give an instance of the infection which appears to show xenoparasit'sm. When the surface of a plant to be inoculated is previously rubbed, the basal cell of trichomes is often broken. The infection takes place predominantly in the cells just surrounding it (Fig. 5, *B*, *C*). Without doubt the chemotactic substance is given off from the broken cell much more than from the intact one. Some of the attracted swarmcells advance into its cavity and proceed to invade the adjacent living cells through their lateral wall.

We speak of the wound parasite when the fungus is considered to get a foothold for infection by obtaining nutrient substances from the dead tissue on the wound surface. So that, according to MÜNCH's subdivision of the disease-producing fungi, the wound parasite is included in the perthophyte. *Olpidium* is MÜNCH's parasite, or, as it is generally called, a true parasite. As evidenced by the inoculation experiments the fresh wound surface is more susceptible than the surface of the intact plant. It is possible that in the field the mechanical injury may occur on the host and facilitates the infection through the wound. For many fungi the outer wall of the epidermis is an obstacle for attacking the plant and the wound surface may be subject to an easier infection. Accepting MÜNCH's distinction between the perthophyte and the parasite we feel it inappropriate to use the term "wound parasite" without limiting its meaning. In the case of *Olpidium* the wound infection is facilitated on account of an easy diffusion of potassium from the exposed surface of the internal cells but not on account of the presence of an available food on the wound surface.

The nature of the epidermal wall may be different in different species or varieties, so that it is probable that the cell wall of certain plants partakes of the genotypic resistance. Further the wall varies in chemical and physical structure according to developmental stages in an individual plant. Obviously external factors, chiefly moisture and light, exert more or less influence upon the development of the epidermal wall; in general under hygrophilous conditions the wall remains thin and soft, rendering the cell

liable to a ready infection. Therefore, we assume that the resistance induced by the cell wall may sometimes be phenotypic[1]. Thus the hinderance of the cell wall to infection is not stable and in general the wall of young cells is less resistant. On this account, in plants of the affinity group we doubt an absolute resistance that may be assigned to the nature of the cell wall.

(3) Cell contents.

Among various substances contained in the living cell the dissociable potassium compounds may be of first importance for causing infection. Undoubtedly the attractive power of the cell depends upon the amount of these compounds which it gives off to the surrounding medium. As is well known, while the amount of potassium in plants varies according to different species and to different organs or portions of a plant, the character of the soil gives rise to variable amounts in the same plant. As to what relation exists between the amounts diffused from and contained in the cell we cannot say clearly. At any rate we are right to suppose that the amount of potassium given off from the cell may be either sufficient or insufficient to cause the chemotaxis upon the swarmcells existing on the surface of the cell. Whenever the action of potassium is feeble or nil the infection would be rare or impossible.

According to several authors who investigate the distribution of potassium in a plant body, the growing and dividing cells are very rich in potassium. PENSTON (1931, p. 688) states, "In all cases it was found that potassium tended to move out of the older regions, especially old leaves, and to be translocated to the young leaves and meristems where its presence apparently led to further growth". On this ground the non-infection of the older portion of the host plant should not be ascribed merely to the thickening of the cell wall, by which, as already pointed out, the outward diffusion of potassium is prevented, but a marked diminution in the potassium content in the old cell would play a greater part in causing resistance. In the course of development of the epidermal cells in general and the internal parenchymatous cells in arboreous plants the thickening of the wall and the diminution in the potassium content may take place simultaneously. Hence it appears likely that their susceptible condition is changed into the resistant one sooner than in other cells which remain thin-walled for a longer period.

1) "Phenotype" is used here in the same sense as the "plastotype" of PRELL (Studia Mendeliana, Brunae, 1923, p. 316).

A consideration of the relation between the resistance and the potassium manuring would be of some interest. On the theoretical ground it is maintained that a cell rich in potassium may attract the swarmcells strongly and may be infected heavily. Therefore, if the potassium manuring gives any effect upon the degree of susceptibility of plants, it should be, as far as our *Olpidium* is concerned, that the potassium shortage gives rise to the resistance to infection. A future research along this line seems to promise results in favour of this view.

In some plants it is possible to assume that, while potassium is diffusing and causing the positive chemotaxis to the swarmcells, some other substances exerting adverse action upon them may come out of the cell. In this case the infection is undoubtedly impossible. The resistance shown by *Cassia mimosoides* var. *nomame*, *Gleditschia*, *Lycopersicum*, *Caesalpinia*, *Dahlia*, and *Calystegia* appears to afford the instances.

Regarding the chemical nature of the cell sap as having some bearing upon the resistance a consideration of acidity has already been given in the preceding pages and independency of the resistance upon hydrion concentration of the cell sap has been explained. For the same reason there is no theoretical evidence as to a direct influence of the chemical constituents of the cell sap upon the resistance to such a fungus as *Olpidium* which entirely relies for existence upon the living protoplast. This may appear to hold true in the haustorial parasites, since in relation to the host cell, or the sap, the haustorium and the intracellular parasite are considered to be similar as may be seen from the investigation of haustoria by RICE (1927, p. 111).

The account given above concerns with the resistance to infection. After establishing the infection the resistance may further be presented when the fungus is unable to develop in the invaded cell. The inhibition of the fungus development may be assignable to the interaction between the fungus and the host plasm. As far as the study on the living material extends, the disorganization of the fungus body is always accompanied by the disintegration of the host plasm. As to which, the fungus or the host cell, precedes the other in this process the observations are not yet accurate. From the general feature presented by such host cells in several plants it is assumed that, as observed in *Synchytrium* (KUSANO, 1929 b), the physiological activity of the fungus causes sooner or later some disturbance upon the organization of the host plasm, and in turn the fungus, being interrupted of the nutritive connection, is brought to death. In *Impatiens*, *Chenopodium*, *Taraxacum*, *Physalis*, and *Cissus* the disorganizing fungus bodies show but

little growth, so that the effect of uncongeniality of the host plasm with the fungus seems to be presented soon after the entry of the fungus. They grow a little in potato cells (Fig. 8). In *Phaseolus angularis* some of the host cells are disorganized after the fungus attains a considerable size. Hence we may state with some certainty that the resistance of the host plasm to the detrimental action of the fungus is presented in varying degrees according to different plants.

Upon consideration so far attempted we may explain the nature of resistance as follows:

In the plants showing the affinity group the problem of resistance arises first on the meeting of the swarmcells with the living cell of the partner. The resistance of the cell depends upon the characters of the wall, the diffusible chemical substances, and the protoplast. To make the matter comprehensive the wall is denoted by *A* when permeable to the potassium compounds and *a* when impermeable or less permeable, the other chemical substances by *B* when indifferent to the swarmcells and *b* when injurious or repulsive, and the protoplast by *C* when congenial with the invader and c when uncongenial. Then, throughout the plants of the affinity group the different types of the cell as related to the parasite may be represented as follows:

Cell	*Infection*	*Development* *
ABC	+	+
ABc	+	—
AbC	—	+
Abc	—	—
aBC	—	+
aBc	—	—
abC	—	+
abc	—	—

As the small letters, *a*, *b*, and c, express the resistant factor, we see that only the cell *ABC* deserves the host, while all others are resistant to infection or to the fungus development.

It may be noticed that in every plant the cell is liable on becoming

* Resistance to infection and to the fungus development is denoted by — and susceptibility by +.

old to change its wall from A to a and to diminish in the potassium content, so that the degree of susceptibility of each cell is not constant. Moreover, the distinction between B and b, or C and c may not be always accurate and they vary as to their degree in different plants. Hence it is easily conceivable that the resistance due to the character of the chemical constituents and of the protoplast would likely be exhibited in varying degrees according to plant species. To elucidate the real nature of the protoplast as expressing C or c appears, at the present state of our knowledge, to be beyond the reach of our investigation. Even the distinction between C and c cannot be made in the cell whose wall and chemical constituents are expressed respectively by a and b. As to the chemical constituents concerned with the resistance to infection there remains much to be investigated in the light of chemotaxis. Indeed they would be extremely variable not only qualitatively and quantitatively but also in the action upon the fungus according to the developmental stages and growing conditions of an individual plant, and according to larger or smaller systematic group of plan s. At any rate the fact that the living cells of 63 out of 81 species examined (included in 16 out of 20 families) are susceptible to infection justifies the conclusion that most of various substances assumed to be found in 81 different plants do not give any interference with the chemotactic behaviour of the swarmcells towards potassium compounds and may be expressed by B according to the designation given above.

In this place we may consider the problem of resistance relating to other fungi, in which the infection is caused by the swarmcells. As already mentioned, *Rhizophidium, Olpidiopsis, Synchytrium*, and *Plasmopara* show each a specific chemotactic response to substances widely occurring in plants. On the same ground as announced in the case of *Olpidium* the affinity group in these fungi is considered to be very large. Nevertheless they occur in nature only on particular plants, viz. *Rhizophidium pollinis* usually on the pollen grains of conifers, *Olpidiopsis saprolegniae* on the mycelia of *Saprolegnia, Synchytrium minutum* on *Pueraria*, and *Plasmopara viticola* on some species of *Vitis*. It is supposed that the resistance of most plants in the affinity group is presented in various ways as mentioned in *Olpidium*; in some the diffusion of the stimulant is feeble on account of its being poor in the cell or because the cell wall is thicker or otherwise; in others certain substances exerting adverse action on the positive chemotaxis is given off from the cell; and in still others the protoplast may be

uncongenial with the invader. MÜLLER (1911) observed that the root hairs of some plants or the algal filaments are infected by *Rhizophidium pollinis* when killed, and even the infection of the proper host, the coniferous pollen grains, is heavier when killed by heat. It is probable that the diffusion of the stimulant is facilitated on killing the cell, as I have ascertained in the present study. From ARENS' (1929) investigation on *Plasmopara* it is known that the leaf tissue of the plants which are able to attract the swarmcells may contain the repulsive substance. Therefore, as already remarked while discussing the parasitic affinity, the plants in the affinity group may become the host, if the attraction of their cells is not prevented either by obstacles involved in the anatomical characters or by any of the chemical constituents of the cells, and further if their protoplast is congenial with the invader. On this assumption we need not conceive that any specific substance causing the infection is monopolised by the susceptible plants. On the contrary attention should be given in susceptible plants to the absence of a special substance acting as a preventive of the infection and in resistant ones to the presence of such substance.

Referring to the Archimycetes we find that *Ligniera Junci* (Schw.) Maire et Tison and *Synchytrium aureum* Schröt. are plurivorous. According to COOK (1926) *L. Junci* invades the roots of a number of aquatic and semi-aquatic Phanerogams. RYTZ (1907) reports that *S. aureum* occurs on more than one hundred species of Phanerogams. There is but little doubt that chemotaxis of their swarmcells is concerned with the infection. Presumably it may be stated that the chemical stimulant widely occurs in higher plants, resulting in a large affinity group and that various diffusible chemical constituents of all susceptible but not of the resistant plants would not be repulsive or injurious to the swarmcells.

In most of the mycelial parasitic fungi the nutritive connection with the host is established through the haustorium. As RICE (1927, p. 73) rightly remarked, we find analogies in haustoria and intracellular parasites as regards the relation to the living cell of the host. This fact leads us to inquire whether or not the chemical stimulus might be concerned with the formation of haustoria. In spite of failure of any convincing evidence many investigators express the opinion that the infection by the parasitic fungi may be induced by the stimulus of a certain substance secreted from the cell of the attacked plant (ZIMMERMANN, 1925, p. 406; KOOLMAN, 1927, p. 117; FISCHER-GÄUMANN, 1929, p. 275). Coming into accord with this opinion I may extend the view maintained in the case of the intracellular parasites to the explanation of the host relation of the haustorial

ones. Here the affinity group may also be large, but in many plants of the group the living cells contain certain substances acting as preventives *of the entrance of haustoria or, as adequately investigated in the rust* fungi, the protoplast may be incompatible with the entering haustorium. In the rust fungi the chemotropic response is often disputed (ARTHUR, 1929, p. 228, 273) *for the reason that it may be observed in many plants* which are divergent in relationship from the systematic group of the host plants. However, from the view here advanced an assumption of a wide occurrence of the chemotropic stimulant in plants other than the hosts is not inconsistent with the highly developed specialization of the rust fungi. If co-existence of an antagonistic substance, as evidenced in *Olpidium*, is assumed to prevail to a large extent among the plants of the affinity group, we may infer that the range of hosts would be reduced to a narrow limit.

This assumption is suggestive of the nature of specialization in parasitic fungi. The plurivory of *Olpidium* is explained as being indifferent towards various chemical substances given off from the cells in most plants of the affinity group. If any fungus acquires the character to be injured or repelled by most of these substances, its invasion would be restricted to such plants that fail to contain them. It results in what we call specialization. Then the specialization of the fungi would mean that they are specialized in their relation to various substances contained in plants.

We now come to emphasize strongly the great need of investigating the chemotropic nature of the haustorial parasites. The positive or negative chemotaxis of free swimming organisms is highly specific. This may appear to hold true in chemotropism of the haustorial parasites. If the formation of haustoria is proved to be prevented or favoured by the *chemical action of the living cells, and moreover, if the nature of the* chemicals concerned is known, more light would be thrown upon several problems involved in the host-parasite relationship.

4. VIRULENCE.

Each of *Olpidium Viciae* and *O. Trifolii* gives a different pathological effect upon different plants; in some hosts no histological distortion is induced, while in others the abnormal cell formation occurs round a hypertrophied host cell. In pathology the action of the parasite upon the host is usually expressed by virulence or pathogenity. As remarked by FISCHER-GÄUMANN (p. 7, 331), both the parasite and the host are respon-

sible for exhibiting a definite virulence; it is a result of the pathogenetic action of the parasite and the reaction of the host to it.

In producing the typical symptom of disease on the proper host the fungus is reasonably considered to exert a certain stimulus upon the host (the host cell and the surrounding cells). When the same fungus develops in a cell of another plant, such an effect is not produced. This fact appears to admit the statement that in reacting to the same fungus each host exhibits a specific difference. If so, the different virulences of *Olpidium* upon different hosts would exclusively be ascribed to the property of the host. Reviewing, however, the results of experiments we need to consider the condition of the fungus development upon different hosts. On the plants giving the typical symptom an enlargement of the host cell with a corresponding increase of protoplast in amount expresses without any doubt an increasing metabolic activity. We look upon this condition of the host cell as a great aid to the vigorous development of the fungus. Here the mutual action between the fungus and the host cell is accelerated; the vigorously growing fungus may exert a strong stimulus upon the host cell, which in turn may supply the fungus with an increasing amount of nutriment.

When a piece of the parenchymatous tissue from the same plant is inoculated, neither does the host cell undergo hypertrophy nor does the fungus attain the normal size. Without the nutritive connection with the mother plant body the host cell in such an isolated tissue becomes not only weak in metabolic activity in itself but it is also incapable of nourishing the residing fungus. It is evident that the ill-nourished fungus is not able to maintain a normal vitality. I am of the view that under such condition the fungus is not able to exert a strong stimulus to cause the hypertrophy on its host cell.

The feature of the wound infection appears to support this statement. The host cell on the wound surface, not being interrupted of the nutritive resources, is often hypertrophied and the tumour cells are produced round it. Of course the fungus grows more vigorously and larger than on the piece of the isolated tissue.

Thus we see that the same fungus brings about different virulences on the same host under, as it appears, different nutritive conditions of the host cell. I maintain that it is an expression of the difference in the intensity of stimulation by the fungus upon the host. The intensity depends upon the vitality of the fungus, which is in turn controlled by nutrition. The host cell which prevents the vigorous development of the fungus weakens the stimulating action of the fungus.

In a former paper (KUSANO, 1929 a) it has been remarked that the much hypertrophied host cell contains a single large gametangium, while the undeformed host cell is often full of many but small gametangia. We recognize between large and small gametangia a difference of vitality and *the action of small gametangia is too feeble to cause deformation on their* host cell.

This explanation can be extended to the case of different virulences *observed in different combinations of both species of Olpidium* and several artificial hosts. Though the fungi are shown to be congenial with the protoplast of the host cell of various plants and can establish the complete *development, the nutritive condition cannot be similar in all of them.* RAINE's (1922) review of literature and his own study strengthen our belief that the vigour of the parasite is proportional to the vigour of the host. *In the hosts, which allow a vigorous development of the fungus, the stim*ulus exerted by it would be strong and the reaction of the host cell is expressed on promoting the metabolic activity. In other hosts, which are *less beneficial to the fungus, the stimulating action of the latter is less* intense and the activity of the host cell is promoted in a less degree.

About the different degrees of the pathologic reaction of potato varieties *to the action of Synchytrium endobioticum* KOHLER (1925, p. 288) proposed a similar view: "Da der parasit sich beim empfänglichen Typ kräftiger entwickelt, gehen von ihm auch kräftigere Reize aus, die Gallen werden grösser". *He thus considers that the virulence of the parasite depends* upon its vitality. FISCHER-GÄUMANN (1929, p. 185, 203) also consider it very probable. As to the relation to the host, *Synchytrium* and *Olpidium present a great similarity. In the nature of virulence both parasites appear* to show also a close resemblance. I am of the opinion that the virulence is not concerned with the resistance of the host but the less degree of the *reaction of the host to the parasite is due to a weak stimulus* exerted by the parasite. This weakening is caused by the depression of its vitality, for which the physiological condition of the host cell is responsible.

This condition of the host may be genotypic, so that on a definite host (fungus carrier) the development of the parasite is always not vigorous. It may also be phenotypic, so that a host plant, which under normal conditions favours the vigorous development of the parasite, hinders it under other circumstances and renders the parasite unable to exert a stimulus strong enough for promoting the activity of the host cell.

Therefore it is questionable whether the different effects of O. Viciae and *O. Trifolii* upon the same host, for instance, *Vicia Faba* or *Pisum*

sativum, may be ascribed to the different natures of stimulus which they exert. Rather, we deem it possible that both fungi exert the same stimulus but in different degrees (weak and strong). This difference in degree is caused by the difference of adjustment regarding the nutritive connection of both fungi with the same plant. It depends upon the hereditary characters of each fungus.

5. Conclusion.

Recapitulating what has been discussed in the preceding pages we may make the following statement:

Olpidium Viciae and *O. Trifolii* have the property of being chemotactically responsive to K-ion. Potassium occurs universally in higher plants localized in cytoplasm and cell sap. On this account the plants exhibit a character to attract the fungi. Thus by the agency of K-ion the parasitic affinity is established between the fungi and plants. The affinity may be strong or weak according to whether the amount of K-ion given off from the plants is large or small. It is assumed that the strong affinity results in a ready infection.

If other chemical substances contained in the living cells of each plant are chemotactically indifferent or not injurious, the plant may be infected. If they cause negative chemotaxis or are injurious, the fungi cannot approach and invade the cells.

The cell wall acts as a barrier against establishing affinity. To exert an attracting action upon the fungi, potassium in the cell must diffuse through the wall on the one hand, and to attain the centre of attraction (cytoplasm) the approached fungi must perforate the wall on the other. Therefore, the nature of the cell wall, physical or chemical, has an important bearing upon ease or difficulty of infection. Under natural conditions the epidermal cells, the outermost living cells of the plant body, are subject to invasion, so that the character of the epidermal system, which varies greatly according to individual species, besides being affected in a certain measure by external conditions under which the plant grows, evidently controls the infection.

Attaining of the fungi to a plant cell appropriate for invasion is connected with the growth form, the habit, and the histological character of the epidermal system of that plant. In certain plants some of these characteristics may prevent the fungi from attaining to the host cell. Obviously the natural host is free from such a hinderance. If this is eliminated by artificial means, then the plant may be infected.

On establishing the infection the protoplast of the invaded cell turns from the centre of attraction to the nutrient medium for the fungi. Its metabolic products would be different qualitatively or quantitatively in different plants, or in different cells of a plant under different or same *external conditions. Hence it should not be assumed that the nutrition of the* invader is wherever and whenever under favourable conditions. In some host cells the invader may develop less vigorously and be less virulent. In extreme cases the invader may not be able to secure the nourishment, if it causes a disturbance of the organization of the host plasm.

Thus the parasitic relation of *O. Viciae* and *O. Trifolii* to higher plants may be variable in its intimacy and in this respect the plants may be grouped as follows:

(1) The affinity group. It comprises all plants in which the cell contains potassium compounds and is able to give off K-ion. To other plants the fungi are indifferent to infection (immune by ZIMMERMANN, KÖHLER, etc.).

(2) The range of hosts. If the plants in the affinity group contain any substance acting repulsively to the invading fungi or if their protoplast is uncongenial with the invader, the host relation is not established. They are genotypically resistant. All others may become the host. We are not yet decided how wide the range of hosts extends in the affinity group, in other words, how many plants in the group are genotypically resistant (genotypically immune by APPEL).

According to the manner of reaction to the invading fungi we may distinguish two types of hosts, viz.

(a) Typical host. It produces tumours by the stimulation of the fungi. We may say that the plant is susceptible to the attack of the fungi and at the same time to the disease caused by them.

(b) Fungus carrier. It shows no external symptom of affection, though the fungi may complete the development. Such a plant may be said to be susceptible to the attack of the fungi but is resistant to the disease (immune by FISCHER-GÄUMANN).

Further we may distinguish two kinds of hosts according to whether the infection is obtained easily or with difficulty.

(c) Artificial host. Due to some causes it is not affected in nature. If these causes are artificially eliminated, the infection is readily obtained and the fungi succeed in development. We may say that the artificial host is phenotypically resistant (phenotypically immune by APPEL).

(d) Natural host. In this group is comprised the plants, upon which

the fungi may occur in nature. Practically they are recognized as producing the typical symptom of disease. The fungus carrier may possibly occur in nature, though it may not be detected. The range of the natural hosts is not exactly known. It may perhaps be wider than is at present recorded.

VIII. Summary.

1. *Olpidium Viciae* and *O. Trifolii* which are found in the field only on *Vicia unijuga* and *Trifolium repens* respectively are ascertained by the inoculation experiments to develop on a large number of plants comprised in the Leguminosae and other families.

2. The infection of living cells is obtained in 63 among 81 examined species (16 among 20 families) of Phanerogams.

3. Among the recognized host plants are distinguished two types with respect to the mode of their reaction to the attack of the parasites. In one type the formation of a tumour resulted, while in the other type no sign of infection is recognized externally.

4. The host of the second type may function as the fungus carrier. Such a plant is said to be susceptible to the parasites but resistant to, or immune from, the disease.

5. The hosts showing the first type to *O. Viciae*, viz. *Vicia unijuga*, *V. Faba*, and *Pisum sativum*, are to *O. Trifolii* of the second type. Reciprocally the plant expressing the first type to *O. Trifolii* is in the second type to *O. Viciae*.

6. The effect of the parasites upon the host plant is similar whether the epidermis or the internal cells on the wound surface are infected.

7. *All parenchymatous tissues from various plants which are liable to* infection induce an apparent positive chemotaxis to the swarmcells (gamete and planozygote). Also the juices of these plants are ascertained to contain the chemotactic substance.

8. Of several water-soluble chemical substances examined only potassium compounds are found to induce the positive chemotaxis on the swarmcells of both fungi. K-ion is an active component.

9. The presence of potassium in the living cells (cytoplasm and cell sap) is considered to establish a close affinity between the parasite and the plant. The wide range of hosts can be explained as being due to a universal occurrence of potassium in higher plants.

10. From the plurivorous nature of *Olpidium* we induce that the

various substances secreted from the living cells of numerous susceptible plants do not exert a repellent or injurious action upon the approaching swarmcells.

11. Young internal cells, vigorous and rich in plasm, in both the shoot and root are most readily susceptible to both fungi. This may be due to the fact that such cells are very plentiful in potassium.

12. Non-infection of a thick-walled cell may be due to the prevention of passage for potassium compounds, and a less susceptibility of an old cell, to a marked diminution of the potassium content, bringing about a feeble attraction for the parasite.

13. Some plants exhibit resistance on account of secreting certain substances which are injurious or repulsive to the approaching swarmcells.

14. In some plants the resistance is induced by the uncongeniality of their protoplast with the invader.

15. The germinating cyst may send out the infection tube or filament into both the living and the dead cells, but the release of its content takes place only when the living plasm is present. It seems to concern with the response of the cyst to K-ion generated in the living plasm.

16. Hydrion concentration in the cell sap does not interfere with the infection of the cell.

17. Many susceptible plants may remain in nature unaffected, since their morphological and anatomical characters, growth forms, or habits prevent the parasite from approaching their susceptible cells.

18. The morphological difference between two forms of *Olpidium* is exceedingly slight, and the range of their hosts exactly coincides. There is, however, a clear distinction between them as regards their effect upon certain plants.

Postscript.

In the course of the work I received through the courtesy of Dr. ED. BAUDYŠ in Brünn the parasitic fungi on *Trifolium* in Tschechoslowakia. The specimens are labelled as follows:

(1) *Synchytrium* (*Olpidium*) *trifolii* (Schröt.) Pass; on *Trifolium repens*; Zebin ad Jičin, Bohemia, 18, VII, 1913; leg. ED. BAUDYŠ.

(2) *Urophlyctis bohemica* Bub.; on *Trifolium repens*; Rytirov u Koutü v Jeseniku, Moravia, 16, VIII, 1919; leg. ED. BAUDYŠ.

(3) *U. bohemica* Bub.; on *Trifolium montanum*; Jičin, Bohemia, 25, VIII, 1916; leg. ED. BAUDYŠ.

(4) *U. bohemica* Bub.; on *Trifolium hybridum*; Železnica, Bohemia, 28, IX, 1916; leg. ED. BAUDYS.

The affected spots, being characterized by the formation of a mass of hypertrophied cells, are essentially similar in three species of *Trifolium*. Especially in *T. montanum* they are larger and more conspicuous, while in *T. repens* they present, like those found in Japan, a less remarkable appearance in particular in dried specimens.

The microscopic examination reveals that all the specimens show the presence of *Olpidium*. On the surface view of the epidermis over the diseased spots an unmistakable indication of the occurrence of this parasite is the empty gametangium, one to several in a brownish host cell. The gametangium wall remains distinct and the outer wall of the host cell is provided with one or more clear pits, often double contoured.

They represent the exit beaks of the gametangium or pores made by them on the wall. In the whole aspect the gametangium precisely accords with that described and figured in *Olpidium Trifolii* (KUSANO, 1929 a). In the diseased spots, though not always, the resting cells are found, often together with the gametangia in the same host cell. Treating with chloral hydrate we can clearly distinguish the thin epispore, swollen thick mesospore, and distinct thin endospore. Thus in the structure of the membrane the resting cells exactly coincide with those of *O. Trifolii* (KUSANO, 1929 a). The resting cell of *Urophlyctis* is characterized by being flat or depressed on one side, but that one I examined is spherical or ovoidal. In the cells of some diseased spots mycelia are visible, sometimes in a form of parenchyma. Any sign of their connection with the gametangia or resting cells above mentioned is not observable. Perhaps they represent any accessory fungus. It is therefore concluded that all specimens given above represent *Olpidium Trifolii* Schröt.

It may be remarked that the diseased spots in *T. montanum* appear to be similar to those described and figured by BUBÁK (1902) as being produced by *Urophlyctis bohemica* Bub. However, judging from his figure showing the form of the resting cells as well as their distribution in the tissue of the diseased spot, it is at once clear that the fungus I examined unquestionably differs from that given by BUBÁK. I am convinced that *T. montanum* may be attacked by both *Urophlyctis* and *Olpidium* producing the symptom of disease very similar to each other. However, I suppose that the diseased spots caused by both parasites would likely reveal certain differences in the external appearance or in the histological structure, if the living materials are closely examined.

Referring to a paper of MAGNUS (1902) who attempted to establish *U. Trifolii* (Pass.) P. Magn. and to identify *U. bohemica* Bub. with it, it seems that *Urophlyctis* and *Olpidium* may occur on the same plants. He expresses the view that SCHRÖTER's *Olpidium Trifolii* might have been established on a combination of the resting cell of *Urophlyctis* and the gametangium of *Olpidium*.

The occurrence of *Olpidium Trifolii* on *Trifolium montanum* being now ascertained with exactness, I emphasize that it is most desirable to carry out a closer comparative study on the symptoms of disease caused by *Urophlyctis* and *Olpidium*.

Dr. BAUDYŠ' specimens are very valuable in widening the range of the natural hosts in *Olpidium Trifolii* which has hitherto been known only on *Trifolium repens*. In the present report the artificial infection of other species of *Trifolium* has been ascertained, and our view as to the possible occurrence of the fungus in the field on various species of *Trifolium* is thus justified by the material from Tschechoslowakia.

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Dormancy in the Summer Sorus of Synchytrium.

By

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With two Text-Figures.

1.

Synchytrium hibernates typically in the form of the resting cell (TOBLER, 1913), but among the species occurring in Japan *S. minutum* (Pat.) Gm. (*S. Puerariae* Miy.) and *S. aecidioides* (Pk.) Lagh. (*S. decipiens* Farl.) form, as far as known, only the summer sorus. The gametangia[1] in the sorus, as in all other species, are thin-walled and when exposed to dry air collapse to die sooner or later. Hence my attention had many years ago been drawn to the mode of wintering of these fungi, and in a study on *S. minutum* (KUSANO, 1908) the following fact came to light. The sori developed in the cortex of the vines of *Pueraria triloba* Mak. (*P. Thunbergiana* Benth.), lying deep in the tissue of the tumour, remain undehisced during the year of formation. On the approach of winter the vines perish, leaving, however, the lower and older portion alive. In the beginning of the next vegetative season, when the winter buds upon the surviving portion of the vine are about to unfold, the tumour bursts in virtue of its tissue tension and on this occasion the gametangia are forcibly dispersed in a pulverous form. These gametangia are the origin of the primary infection of the new vines. At this period the sori on the dead portion of the vine are found already bleached and dried, remaining permanently inclosed in the tumour tissue.

In the case of *S. aecidioides* the host plant, *Falcata japonica* Kom. (*Amphicarpaea*), is annual and no portion of the vegetative organs is left alive for the next year. The sori developed upon its vine, successively from the lower to the upper portion, dehisce on attaining maturity, giving

1) In *Synchytrium* the summer sorus in general is considered to be produced by the parthenogenetic development of the gamete (KUSANO, 1930).

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to the tumours an appearance of empty cups of *Aecidium*. It shows that the same manner of hibernation as observed in *S. minutum* is not expected. Therefore, the question of whether the summer sori truly hibernate or we miss the resting cells, actually but rarely formed, has up to now remained in obscurity.

Having occasion in the late autumn of the year 1930 to observe *S. aecidioides* upon the host plant which was somewhat suffering from frequent frost, my attention was attracted by the occurrence of numerous small hemispherical bodies, which were distinctly black and shining on the greenish surface of the plant. Since *S. aecidioides* is known always to produce orange-coloured tumours, my first impression was that these questionable bodies were produced by some other parasitic fungus. Submitting them to microscopic examination for identification I was struck by the fact that they represent in fact the tumours containing the orange gametangia of *S. aecidioides*. Towards the close of the winter season these tumours remained in the field still intact upon the entirely dead and dried-up vines. Though the gametangia inclosed in them were pulverous and polyhedral from drying, their contents retained the same appearance as that assumed by the fresh meterial upon the living host. Introducing them into water at an indoor temperature (15°C.) they soon swelled into a spherical form and after some hours liberated active gametes into the surrounding medium. It is evident that the gametangium sori retained their vitality over the winter.

As a rule the wintering sori occur, not on the lower and older, but on the upper, lately grown portions of the vine, and they are not found at any other period than the late autumn. Taking into consideration the fact that the fungus does not attack the older portions of the host, such an orientation of the wintering sori upon the vine and the period of their appearance as just mentioned leave but little room for doubt in concluding that they represent the last stage of fungus development in the year. This conclusion has been fortified by observing the development of the sori during the month of October of the next year.

The tumour, in which the wintering sorus is developed, does not differ in structure from that which contains the usual one, and over the sorus there occur only one or two layers of stretched or compressed tumour cells inclusive of epidermis (Fig. 2, *B*). All tumour cells are deeply brown throughout the wall and cavity. Also the sorus wall, which is usually hyaline, is here coloured brown and affords a compact and tough appearance. As a whole, with the exception of the discolouration, the sorus and the

tumour reveal nothing peculiar in structure to make them distinctive from those developed at preceding periods.

The brown tissue of the tumour is less liable to corruption than the other parenchyma of the host. In water, while the cortex of the dead stem softens and collapses, the tumour remains compact and is easily isolated in an intact form from the rotten cortex. It seems that the durability of the tumour tissue would make its protective action upon the sorus more effective. In this sense the tumour tissue is comparable with the brownish host cell of the resting cell in other species of *Synchytrium*. As described in *S. fulgens* (KUSANO, 1930), when the resting cell is full grown, the host cell begins to turn brown throughout the cavity and wall. When the surrounding epidermal cells are about to decay, it withstands corruption and remains as a case for the resting cell. The brown colouration, being caused by the fungus, would in all probability follow the same process in both cases. However, whether its significance upon the dormant fungus body is similar or not remains questionable.

2.

In *S. minutum* we pointed out that the wintering sori may dehisce by the aid of the expanding action of the surrounding living tissue of the tumour. In *S. aecidioides* the host tissue round the sori, being already dead, is incapable of participating in this action. It may naturally be questioned what mechanism is concerned with the dehiscence of the wintering sori. To answer this question the following experiments were carried out.

(1) At the commencement of March the material was collected from the field and after immersion in water for a while was placed in PETRI's dishes. One of them was kept at a temperature of 18°C. and other below 10°C. On the next day some sori in both dishes were found dehisced. The number of the dehisced sori increased day after day, but the rate of increase, as shown in Table 1, was greater at the higher temperature. The dispersed gametangia were much swollen and no more polyhedral. In water they liberated gametes in two to three hours.

There were a number of the sori remaining undehisced after 20 days. The gametangia have been ascertained to retain the polyhedral form, being discoloured or containing disintegrated contents.

(2) In November dead affected vines were brought from the field to the laboratory room and kept in PETRI's dish till March of the next year. To keep them from extreme dryness in the heated room during winter

drops of water were from time to time given to the dish. On soaking the vines with water the dehiscence of the sori, beginning in an hour, was taking place day after day. After a week the gametangia taken from undehisced sori were tested for germination. Some sori furnished the germinative gametangia, while others did not. This showed that some sori were already dead, perhaps from drying, while some retained viability still remaining undehisced. Examined after two weeks a majority of the sori were found to have dehisced.

TABLE 1.

Showing the number of dehisced sori on successive days at different temperatures.

No. of vine piece / No. of day	I	II	III	IV	V	VI
		(10°C.)			(18°C.)	
1	14	2	1	6	8	0
2	15	6	2	16	16	0
3	15	8	2	22	16	9
5	17	8	2	48	55	25
6	18	8	2	88	66	65
7	18	8	2	89	69	73
8	18	8	2			
		(15°C.)				
9	21	9	2			
10	26	11	3	100	76	81
		(18°C.)				
12	57	25	7			
13	106	39	11	105	76	86
20	125	63	29	110	83	86
No. of undehisced sori	5	6	13	0	5	0
Total number of sori	130	69	42	110	88	86

(3) The material which remained outdoors till 20, May was wetted. Some sori dehisced in from one to two hours after getting water. A similar result was obtained in the material which was brought from the field on 15, March to a chamber at 10°C. and preserved there till 12, July. The number of the dehisced sori was progressively increased on succeeding days as in the preceding experiments.

(4) The material collected in the late autumn was preserved as a herbarium specimen after completely drying. Treating it in March of the next year as before, no sorus came to dehiscence. Dissecting undehisced sori orange gametangia were obtained. They appeared to be alive but in water remained polyhedral, being never swollen. Germination did not take place and in a few days they were bleached and disorganized. Without doubt all sori have lost their vitality on drying.

(5) The above observations have all been made after the dormant period. In order to know the feature of dehiscence in the early part of that period, the material was brought from the field to the room in the beginning of December. The host plant had already perished by the end of November, and the tumours and vines were apparently dry. Keeping the material sufficiently moist in PETRI's dish at a temperature fluctuating between 10° and 17°C., no sorus was dehisced even after 10 days. The gametangia were taken from the closed sori of the same material, which had been kept in the dish for a week, and were introduced into a hanging drop of water. Exposing them to 15°–17°C. germination did not take place at the usual rate but was delayed as much as two or three days. Probably a certain interval of time was required for the gametangia just entering the dormant stage for restoring their physiological activity when brought to a germinative condition.

After two months only a few sori on the same material were found dehisced. At this time the healthy tissue of the host became somewhat softened but the tumour tissue still remained rigid. The gametangia taken from the closed sorus germinated at 17°C. in one or two days.

Another specimen was brought from the field at the end of January and kept moist at 17°C. After a few days none of the sori came to dehiscence, but the gametangia taken from them were able to germinate in a day. There is reason to believe that the tumour tissue, remaining in the field undecayed, withstands the breaking action of the inclosing sorus which is about to swell by the absorption of water.

The sori on the same material dehisced to a large percentage after from ten to twelve days.

From the result of the foregoing experiments we see that the sori inclosed in the dead tissue of the host dehisce by their own power. When a critical temperature is attained in the spring, the gametangia begin to absorb water to such an extent that their swelling action overcomes the tension of the sorus wall and the overlying tumour tissue. In all probab-

ility the water-absorbing substances are generated at this time by the restoration of the metabolic activity in the gametangia. It may be true that the dried sori absorb water and more or less swell without the generation of such special substances. However, taking the undehiscence of dead sori into consideration, it is reasonable to assume that to exert a strong internal pressure sufficient to cause dehiscence the sorus must produce certain osmotic substances, which had been absent during the dormant period.

It is clear that in dehiscing the swelling action of the sorus must overcome the resistance of the sorus wall and the tumour tissue overlying the sorus. As to why the dehiscence takes place at varying periods the withstanding strength of the tumour tissue may be taken as an essential cause. The dehiscence of some sori as early as one or two hours after giving water would be due to the less resistant condition of the decaying tumour tissue. On the other hand its retardation for a week or two indicates that the tumour withstands the breaking action of the swelling sorus. Generally the weathered tumour tissue softens easily in water and decomposes so as to be readily broken. This process would proceed at different rates in different tumours. It appears very probable that the undehiscence at a low temperature or at an early period of winter is due not only to the suppression of physiological activities in the sorus but also to the rigid condition of the tumour tissue. Therefore we need not assign the divergence in the period of dehiscence to different strengths of the osmotic pressure in individual sori. Such being the case, we would rather regard the tumour tissue, unlike that in *S. minutum*, as a hinderance in more or less degree to the dehiscence of the wintering sorus.

3.

The conditions indispensable for the summer sori functioning as the wintering form are (1) undehiscence previous to entering the dormant period, (2) protection in particular from drying during that period, and (3) dehiscence at an appropriate period subsequent to wintering. As the most important feature in satisfying these conditions we mention an unusual process of development of the sori in each of both fungi.

To begin with *S. minutum* it is emphasized that the wintering sori take a longer period in completing the development. While the usual forms, mostly occurring on leaves mature in from three to four weeks after infection, those on the stem are not only in general slower in development but are very variable in the maturation period. For instance, some are observed

to dehisce about two weeks later than those on the leaves, while others dehisce after a further two weeks. Besides, there are still younger ones which take more time to attain the dehiscing stage. All these, though delayed in maturation, are nothing but the usual summer sori. The fungus individuals which are destined for the wintering sori are strikingly retarded in development, and when examined after two months each sorus is represented by an orange viscous fluid occupying the lysigenic cavity in the tumour[1]. It is still at a uninucleate or prosorus stage. This has been ascertained at the end of July on examining the tumour formed as the result of the primary infection. The subsequent development proceeds still more slowly. In October the fungus body retains a dense viscous consistence and at the commencement of November the segmentation of the body into the gametangia takes place. Hereafter the retardation of the development seems to be enhanced by the winter season. In January of the following year the sorus is found to be completed, but the gametangia are young remaining polyhedral and being compactly united together. Their full maturity into a pulverous form is attained in February. Thus the wintering sori come to maturity at the close of the winter season, so that, strictly speaking, the dormant period does not exist in them. At this season the tumour tissue and even the entire perennial tissue of the host plant is in a dormant condition and incapable of exerting the expanding action which aids the sorus in dehiscing.

How such an unusually slow development is caused in the wintering sori is not obvious. However, it should be noted that they are all found deeply seated in the tumour tissue. Generally this tissue on the stem is more massive than on the leaves. It is especially so when a considerably large tumour is produced by the coöperation of the aggregated fungus individuals (often 30–50). In this case the orientation of each fungus individual in the tumour tissue is very variable; some occur near the surface while others are shallow- or deep-seated. It is observed that the individuals occurring near the surface develop into the usual summer sori, while the deeply seated ones all become the wintering form (Fig 1). It is supposed that the nutritive relation between the host tissue and the fungus is different in the peripheral and inner portion of the tumonr. Perhaps the inner portion maintains the nutritive activity for a longer duration, enabling the fuugus to prolong the growing stage. It may be due to this condition that

1) That the fungus dissolves the wall of the host cell, and perhaps the surrounding cells, to make sufficient room for its own growth has already been given (KUSANO, 1907, 1909).

the sori attain a larger size, 1 mm. in diameter, while those occurring elsewhere is less than $^1/_3$ mm. As to the cause of the favourable nutrition of the fungus in this portion of the tumour we may point out the great vigour of the tissue. Stimulated by the growing fungus, the tumour is

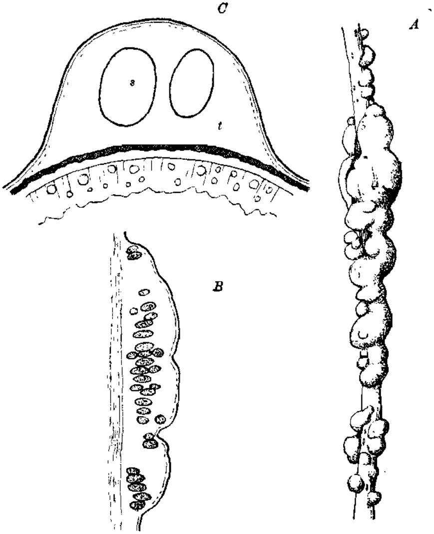

Fig. 1. Wintering sori of *Synchytrium minutum* and tumour produced. *A*, basal portion of the stem of *Pueraria* heavily infected (nat. size); *B*, longitudinal section through a tumour of the same (magnified); *C*, cross section of the stem through a tumour (*t*), in which two prosori (*s*) are developing.

undergoing the multiplication of cells chiefly in the inner portion, and while the peripheral tissue becomes permanent and less active, the inner tissue remains meristematic, being vigorous in physiological activities. Morever, we observe that the lower and basal portion of the stem is grow-

ing in thickness for a longer interval of time, and the tumours developed there attain in general a larger size than those on the upper portion of the stem. As a noteworthy fact the tumour on the upper slender portion of

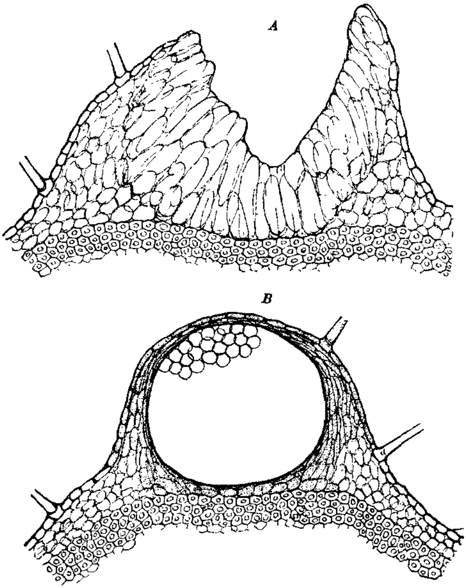

Fig. 2. Cross section of the stem of *Falcata* through the tumour caused by *Synchytrium aecidioides*. *A*, tumour containing a wintering sorus; *B*, tumour, in which a summer sorus is dehisced. ×150

the stem, like that on the petiole, remains small, with a thin layer of the tissue overlying the sori. Most of these sori mature in a shorter period, to function as the summer form. This fact affords an evidence of the exis-

tence of a correlation between the activity of the tumour cells and the maturation period of the fungus.

The case is quite different in *S. aecidioides*. The summer sori developed in other seasons are accustomed to dehisce immediately upon maturity. In a like manner as already described in *S. minutum* (KUSANO, 1907, 1908), two mechanical actions are coöperative. The one is the osmotic pressure of the sori, while the other, perhaps more effective, is a strong pressure exercised by the exanding tumour tissue just surrounding the basal and lateral sides of the sori (Fig. 2, *A*). As to why the same mechanism of dehiscence is not operative in the wintering sori at maturity it is to be specially noted that the death of the tumour tissue is there concerned. The browning of the tumour, as mentioned before, indicates the unnatural death of the tissue, for which the fungus is responsible. It takes place while the host plant, though approaching the end of the vegetative period, is still alive and greenish. With the death of the tumour tissue the fungus is cut off from nutritive resources, and with the close of the growing stage proceeds to develop into the sorus. It follows that at the time the sorus is fully ripe the tumour tissue has already become inactive. In addition, *the season is getting colder and the sorus enters the dormant stage*, as the generation of the osmotic substances is prevented, perhaps by low temperatures.

It remains at present quite inexplicable how the tumour comes to die without dehiscence and prior to the surrounding normal tissue. Probably it may be connected in some measure with the physiological condition of the entire host plant. In the middle of October I examined in the field an affected host plant carrying young sori. They were all of the same age. In a few days the sori on the leaves and young portion of the stem came to dehiscence but those on a somewhat older portion of the stem remained undehisced, and the tumours inclosing them begun to turn brown in the following few days. The portion of the host carrying the dehiscing sori was yet young, vigorous, and turgescent, while that, upon which the undehisced sori occurred, appeared fully developed and less turgid. Perhaps the tumour cells on the older portion became less vigorous at the maturation period of the sori, and, being unable to generate a strong osmotic pressure for dehiscing, proceeded to lose their vitality earlier than the surrounding normal tissues.

The protection of the sorus from drying during the dormant period is easily comprehensible in *S. minutum*, as it is deeply seated in the living tumour tissue (Fig. 1, *B*). In *S. aecidioides* the sorus is almost subepiderm-

al in the dead tumour, lying beneath one to two layers of cells (Fig. 2, *B*). Obviously these cells would play only a small part in protection, so that it appears probable that the sorus itself may be provided with a property of withstanding dryness.

After hibernating the sorus of *S. minutum* dehisces, as mentioned before, by the aid of the expanding action of the living tumour tissue, which becomes operative on the restoration of the physiological activity at the arrival of the vegetative season. In the sorus of *S. aecidioides* the dehiscence is not aided by the host. The swelling action of the sorus may be sufficient to break the sorus wall and a thin layer of the more or less weathered tumour tissue overlying it.

Thus the wintering sori of two fungi present great divergence in several respects; (1) they are found in one case on the lower and in the other on the upper portion of the stem, (2) they are formed in one case at an early and in the other at the last sorus generation, (3) their period of development is exceedingly prolonged in one case but not in the other, (4) they hibernate in one case in the living and in the other in the dead host tissue, (5) they are dehisced in one case by the aid of the host tissue and in the other by their own power, and so on. When we recall the fact that these fungi show a pronounced resemblance in essential points relating to their cytological, morphological, and physiological characters (KUSANO, 1908, 1909), such a remarkable divergence particularly as presented in the mode of hibernating would very likely have been brought about as the result of their adaptation towards the habit of their host plants, the one being partly perennial and the other entirely annual. At any rate these fungi afford a remarkable instance of how precisely the mode of life is adjusted to the conditions of the host plants.

4.

The hibernation of the given fungi in this way casts a clear light upon the sexual problem attached to them. In adequately investigated species it has been ascertained that the resting cell originates from the zygote produced by two copulating gametes (KUSANO, 1930). In *S. minutum* and *S. aecidioides* my efforts to find the resting cell and to observe the copulation of gametes were all in vain. Without an exact knowledge of the mode of hibernating, however, such a negative result of observation has not hitherto been confidently accepted as proving the absence of sexuality. Now, the reappearance of these fungi in the next year by means of the

hibernating summer sori is clearly demonstrated, and we are justified in believing that an omission of sexual reproduction is absolutely established.

In recent year GÄUMANN (1927) attempted to enumerate nine tropical species of *Synchytrium* all on the Leguminosae, inclusive of *S. minutum* and *S. aecidioides*[1]. Most of them are oriental, but in our fungus flora only the last-mentioned two species are recorded, the others being perhaps restricted in distribution to the tropics. As a noteworthy fact they all seem to be wanting in the resting cell, that is, they seem to omit sexual reproduction. Ecologically considered, the absence of the resting cell may be correlated with the tropical habitat[2]. Among these species the extended distribution of *S. minutum* and *S. aecidioides* beyond the tropical regions, for instance, as far as the northern part of Japan[3], would have been possible through their adaptation for hibernation without providing the resting cell. As it appears that the presentation of this adaptation is, as already observed, closely connected with the winter season, these and also the remaining species would in the tropics very likely repeat the generation of the summer sori, not being interrupted by the dormant period. However, as regards *S. minutum* occurring in the tropics on *Pueraria triloba*, it is questionable whether the sori on the stem can attain maturity in so a short period as those on the leaf.

In a former paper (KUSANO, 1930) I expressed the view that the absence of the resting cell in some species of *Synchytrium* is considered to be due to the retrogression of sexuality in gametes. It would follow from this that such tropical species as given by GÄUMANN had been descended by adaptation to the tropical climate from the group[4], which is distributed in the temperate regions, and in which the necessary dormancy is made possible by performing sexual reproduction. On this view an occasional copulation in gametes of *S. psophocarpi* (Rac.) Gm., which GÄUMANN (1927, p. 171), if I understand him aright, seemed to have observed in Java and thought

1) All these were formerly included in either *Woroninella* or the rust fungi. The genus *Woroninella* which was established by RACIBORSKI in 1898 was ascertained by GÄUMANN to be identical with *Synchytrium*.

2) *S. Wurthii* Rytz known in Java is reported as having the resting cell (RYTZ, 1907). Requiring no perceptible resting period the cell germinates upon the living host into the gametangium sorus. As to whether it originates from the zygote or, as illustrated in *S. endobioticum*, it expresses a developmental stage of the summer sorus, in other words, a phase of sexual reproduction, we need further investigation.

3) About their occurrence in Hokkaido I owe to the kind information of Prof. S. ITO, Imperial University of Sapporo, for which I express my deepest thanks.

4) Probably this group belongs to TOBLER's subgenus *Pleiochytrium*, in which the gametes are able to develop parthenogenetically.

› be an abnormal process, may be interpreted as showing that the sex ıaracter is not as yet entirely disappeared from the gametes.

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